



FACULTEIT DIERGENEESKUNDE
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Ultrasonography of Feline Adrenal Glands

ANAÏS COMBES

Thesis submitted in the fulfillment of the requirements for
the degree of Doctor in Veterinary Sciences (PhD)

13 January 2014

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LIST OF ABBREVIATIONS

17OHP	17 alpha-hydroxyprogesterone
2D	two dimensions
ACTH	adrenocorticotrophic hormone plasma concentration
ADH	adrenal-dependant hyperadrenocorticism
AG	adrenal gland
ASD	androstenedione
ATH	adrenal tumor-dependant hyperadrenocorticism
cdH	caudal height
cdW	caudal width
CHUVA	Centre Hospitalier Universitaire Vétérinaire d'Alfort
CI	confidence interval
CKD	chronic kidney disease
Cl	plasma chloride concentration
CORT	cortisol
CRH	corticotropin-releasing hormone
crH	cranial height
crW	cranial width
CT	computed tomography
DHEA	didehydroepiandrosterone
dLAGcdH	difference between pre- and per-anaesthetic ultrasonographic measurements of the caudal height of the left adrenal gland
dLAGcrH	difference between pre- and per-anaesthetic ultrasonographic measurements of the cranial height of the left adrenal gland
dLAGL	difference between pre- and per-anaesthetic ultrasonographic measurements of the length of the left adrenal gland
dRAGcdH	difference between pre- and per-anaesthetic ultrasonographic measurements of the caudal height of the right adrenal gland
dRAGcrH	difference between pre- and per-anaesthetic ultrasonographic measurements of the cranial height of the right adrenal gland
dRAGL	difference between pre- and per-anaesthetic ultrasonographic measurements of the length of the right adrenal gland
ECVDI	European College of Veterinary Diagnostic Imaging
ESTR	estradiol
FeLV	feline leukaemia virus
FIC	feline idiopathic cystitis
FIV	feline immunodeficiency virus

GH	growth hormone
HAC	hyperadrenocorticism
HCM	hypertrophic cardiomyopathy
HPA	hypothalamic pituitary adrenal (axis)
IGF1	insulin-like growth factor plasma concentration
K	plasma potassium concentration
L	length
LA	limit of agreement
LAG	left adrenal gland
LDDS	low dose dexamethasone suppression (test)
LH	luteinizing hormone
MRI	magnetic resonance imaging
Na	plasma sodium concentration
NE	not evaluated, not examined
P	plasma phosphorus concentration
PAC	plasma aldosterone concentration
PDH	pituitary-dependant hyperadrenocorticism
PET	Positron Emission Tomography
PG	progesterone
PHA	primary hyperaldosteronism
PKD	polycystic kidney disease
PRA	plasma renin activity
RAG	right adrenal gland
SAG	sagittal plane
SBP	systolic blood pressure
SD	standard deviation
SHSN	sexual hormones-secreting neoplasia
To	plasma cortisol concentration before dexamethasone injection
To+4h	plasma cortisol concentration 4 hours after dexamethasone injection
To+8h	plasma cortisol concentration 8 hours after dexamethasone injection
TESTO	testosterone
TR	transverse plane
tT ₄	total serum thyroxine concentration
UCCR	urine cortisol:creatinine ratio
US	ultrasound, ultrasonography, ultrasonographic

1 GENERAL INTRODUCTION

This introduction chapter provides an overview of the current literature on the ultrasonographic examination of the adrenal glands in cats.

The first part reports the normal anatomy and physiology of the adrenal glands in cats. According to this knowledge about normal adrenal glands, the scanning technique and then, the normal ultrasonographic appearance of the feline adrenal glands are described. The second part gathers the current knowledge on the ultrasonographic appearance of the adrenal glands in feline endocrine diseases, such as hyperadrenocorticism, hyperaldosteronism, secreting and non-secreting neoplasms. In the last part, the effect of biochemical changes (hypertrhyroidism, anaesthesia, chronic diseases) on feline adrenal ultrasonography is discussed.

Adapted from:

Combes A., Saunders J.H. **Ultrasonographic examination of the feline adrenal glands: a review.** Accepted in *Pratique médicale et chirurgicale de l'animal de compagnie* for publication in 2014.

1.1 ANATOMY AND PHYSIOLOGY OF THE FELINE ADRENAL GLANDS

The adrenal glands are paired, flattened, ovoid organs located in the retroperitoneal space, medial and cranial to each kidney (Figure 1 and 2). The left adrenal gland lies ventrolateral to the aorta, close to the cranial mesenteric artery. The right adrenal gland lies more cranial than the left one, located dorsolateral to the caudal vena cava and caudal to the right diaphragmatic crus. The phrenico-abdominal arteries course on the dorsal surface of the glands, from the aorta ventro-medially to the paraspinal muscles dorso-laterally. The phrenico-abdominal veins course on the ventral surfaces of the glands after draining the paraspinal muscles dorso-laterally and ending in the caudal vena cava ventro-medially (Figure 1 and 3)^{1,2}.

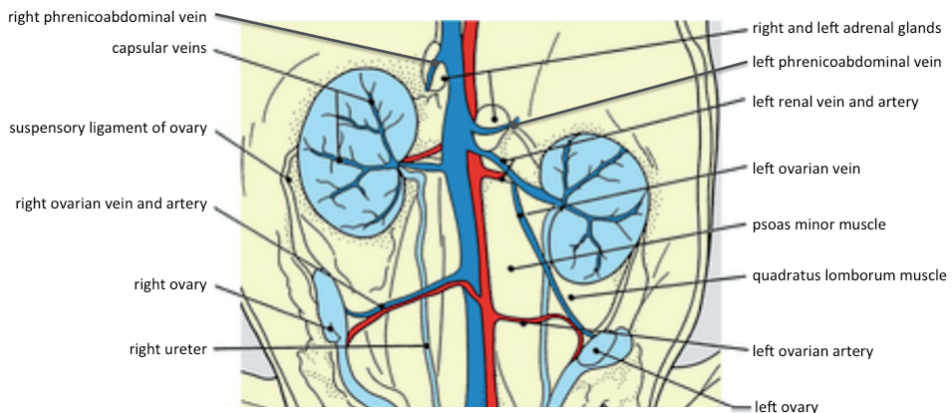


FIGURE 1 ANATOMICAL DRAWING OF THE RENAL AND ADRENAL AREA, VENTRAL VIEW. ADPATED FROM: *COLOR ATLAS OF VETERINARY ANATOMY, THE DOG AND CAT, SECOND EDITION. SH DONE, PC GOODY, NC STICKLAND, SA EVANS, EA BAINES, EDS. MOSBY ELSEVIER, 2009.*

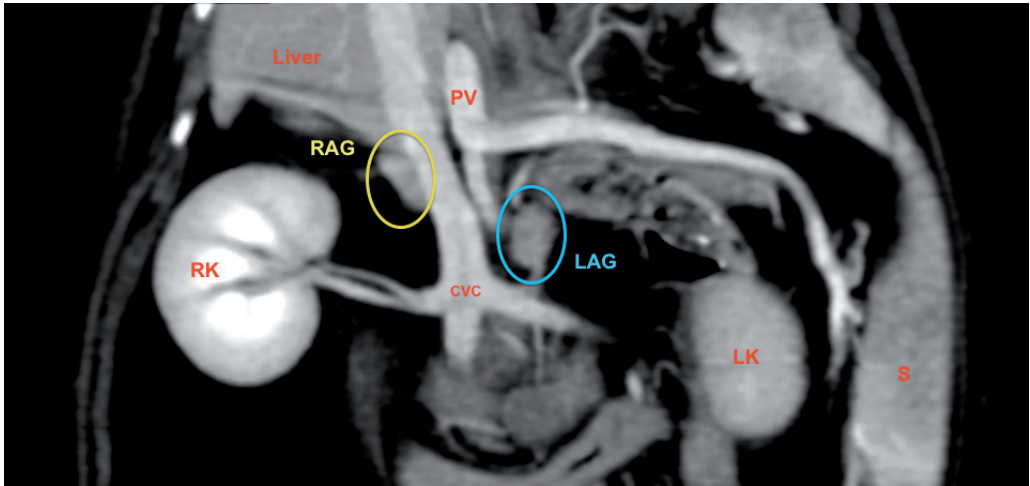


FIGURE 2 MAXIMUM INTENSITY PROJECTION IN DORSAL PLANE FROM COMPUTED TOMOGRAPHIC IMAGES AT THE LEVEL OF THE ADRENAL GLANDS IN A CAT. CVC CAUDAL VENA CAVA, LAG LEFT ADRENAL GLAND, LK LEFT KIDNEY, PV, PORTAL VEIN, RAG RIGHT ADRENAL GLAND, RK RIGHT KIDNEY, SPLEEN.

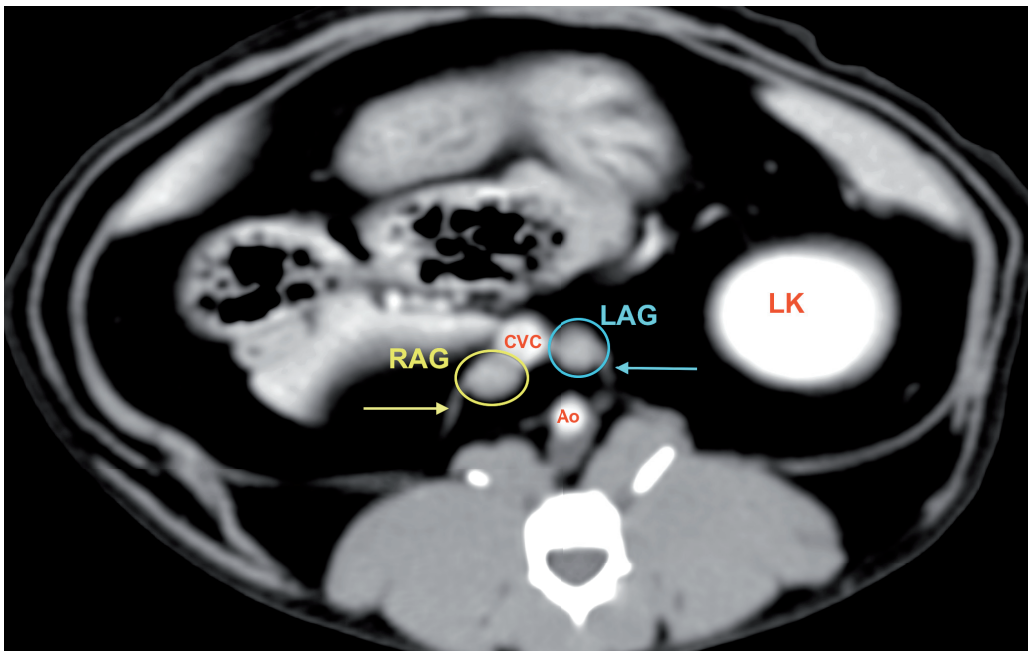


FIGURE 3 TRANSVERSE POST-CONTRAST COMPUTED TOMOGRAPHIC IMAGE AT THE LEVEL OF THE ADRENAL GLANDS IN A CAT. THE RIGHT (YELLOW) AND LEFT (BLUE) PHRENICOABDOMINAL VEINS ARE SHOWN BY ARROWS. Ao AORTA, CVC CAUDAL VENA CAVA, LAG LEFT ADRENAL GLAND, LK LEFT KIDNEY, RAG RIGHT ADRENAL GLAND. THIS PICTURE SHOWS A CAT IN DORSAL RECUMBENCY, LIKE DURING AN ULTRASOUND EXAMINATION, TO COMPARE MORE EASILY CT AND ULTRASOUND IMAGES.

The blood supply of the adrenal gland is provided by multiple sources: the caudal phrenic artery, the renal artery, the first or second lumbar artery, the celiac artery, the cranial abdominal artery, the cranial mesenteric artery and the aorta. The adrenal glands are drained by the suprarenal veins, which join the neighbouring veins³.

Histologically, a thin capsule, consisting of dense connective tissue and smooth muscle fibres, surrounds the gland and gives rise to trabeculae that variably penetrate the adrenal parenchyma.

The adrenal gland is composed of two different tissues: an outer cortex and an inner medulla, of different embryologic origin (Figure 4).

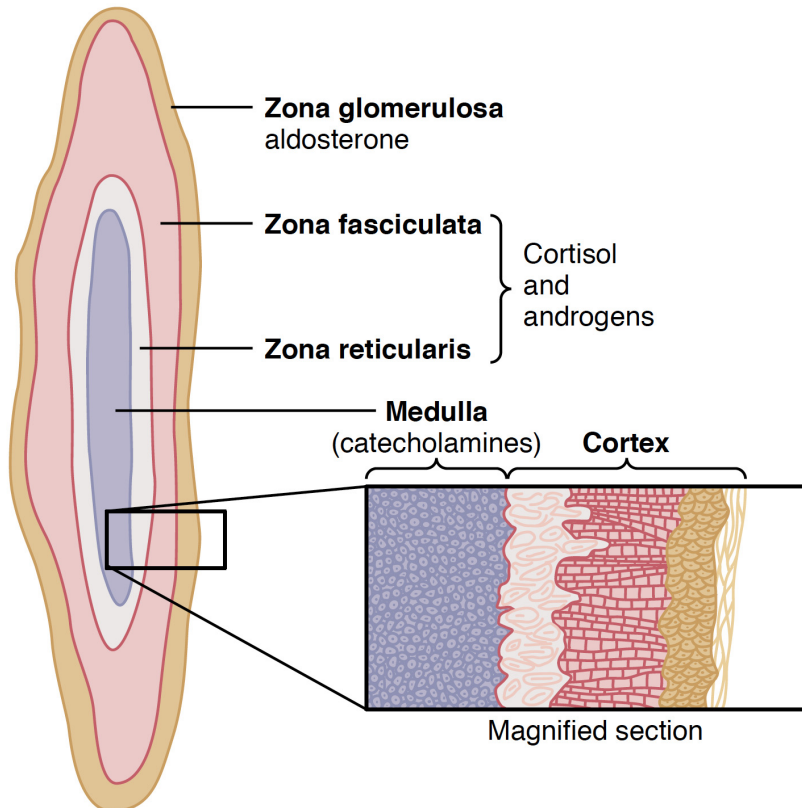


FIGURE 4 SCHEMATIC ANATOMY OF THE ADRENAL CORTICAL LAYERS AND ADRENAL MEDULLA WITH HORMONAL SECRETION. ADAPTED FROM: TEXTBOOK OF MEDICAL PHYSIOLOGY, 11TH EDITION. AC GUYTON & JE HALL, EDS. ELSEVIER SAUNDERS, PHILADELPHIA, 2006.

- The adrenal cortex has a mesenchymal origin. It has the potential for producing and secreting a variety of steroid substances (progesterone, testosterone, cortisol, aldosterone, oestradiol) depending on the enzymes available in the different cortical layers (Figure 5).

It is organized in three layers: glomerulosa (secreting mineralocorticoids like aldosterone), fasciculata (secreting glucocorticoids) and reticularis (secreting glucocorticoids and sex hormones).

- a. Aldosterone promotes potassium excretion and stimulates conservation of sodium and secondarily of water. It raises blood volume and increases blood pressure⁴.
- b. Cortisol affects almost every tissue: gluconeogenesis and glycogenesis by liver and muscle, suppression of peripheral glucose uptake, enhanced protein and fat catabolism, stimulation of erythrocytosis, suppression of inflammatory responses and lymphoid tissue, maintenance of normal blood pressure and counteraction of the effects of stress⁴.
- c. Progesterone is a precursor of other sex hormones, such as oestrogens and androgens⁵. In addition to its effect on the reproductive system, progesterone may simulate the actions of cortisol by competitively binding to the cortisol-binding proteins in the circulation and thus releasing active, unbound cortisol in circulation despite normal total serum cortisol⁶. Some progestins also have intrinsic glucocorticoid effect⁵.

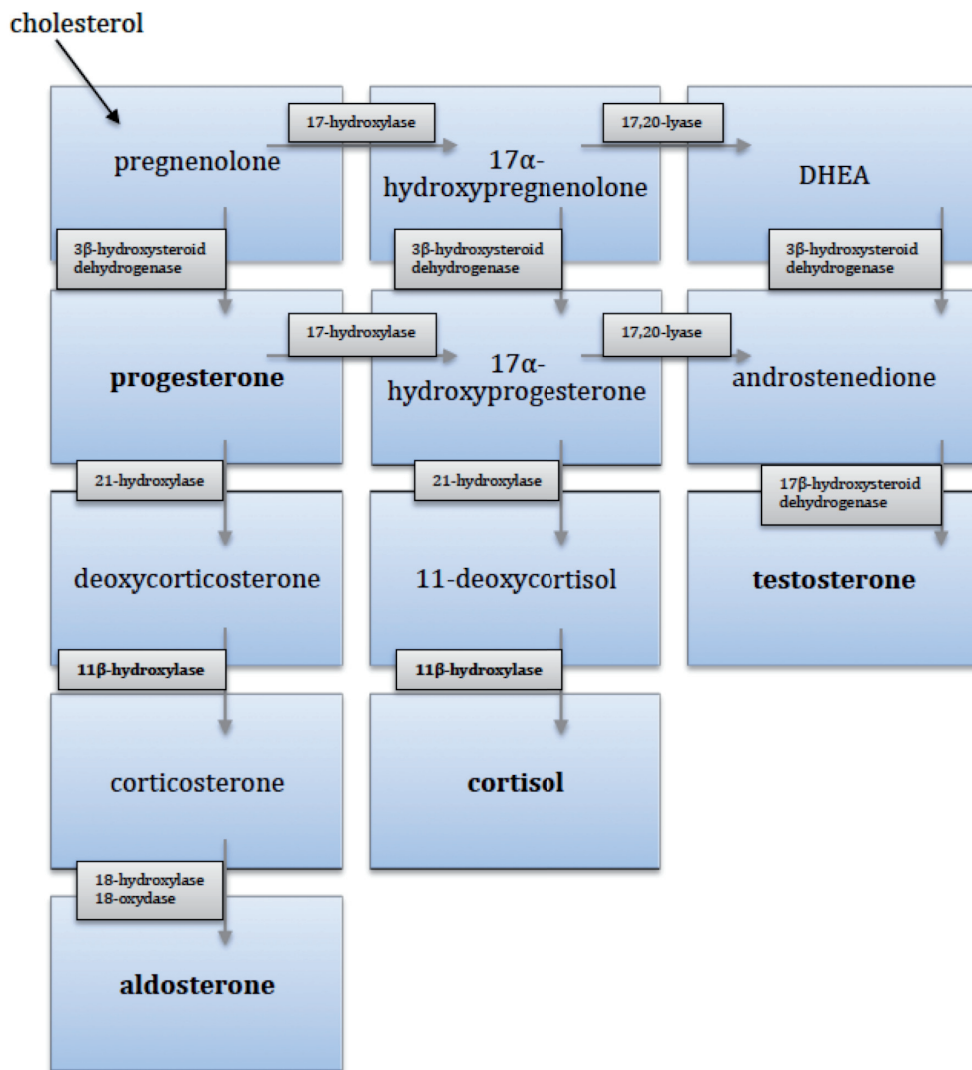


FIGURE 5 STEROID BIOSYNTHETIC PATHWAY IN THE ADRENAL GLAND (STEROIDS IN BLUE BOXES AND ENZYMES IN GRAY BOXES). ADAPTED FROM FELDMAN AND NELSON, 2004 AND KNIGHTON, 2004.

Adrenocortical secretions are regulated by adrenocorticotrophic hormone (ACTH), produced by the pituitary gland. Its stimulatory properties are more important on glucocorticoid secretion than on mineralocorticoid or androgenic steroid secretions. ACTH secretion is controlled by the hypothalamus and central nervous system with corticotropin-releasing hormone (CRH), arginine

vasopressin and other neurotransmitters. Cortisol also has a negative feedback control on ACTH secretion at both the hypothalamic and pituitary level⁶.

- The adrenal medulla arises from neural crest cells and it consists of postganglionic sympathetic neurons without axons. Adrenal medullary production of catecholamines is mediated by acetylcholine released from stimulated preganglionic sympathetic nerve fibres¹. Catecholamines are secreted into the blood stream and represent a sympathetic transmitter through the alpha and beta adrenergic receptors on multiple organs. Examples of sympathetic effects are mydriasis, decreased glandular secretion, vasoconstriction or vasodilation, tachycardia, inotropism, bronchodilation, decreased gastro-intestinal activity, decreased urine output, urine retention, increased blood glucose, lipids and coagulation, muscle glycogenolysis and lipolysis⁷.

1.2 ULTRASOUND SCANNING TECHNIQUE OF THE FELINE ADRENAL GLANDS

The cat can be restrained manually or chemically. However, in a quiet and dark environment such as an ultrasound room, most of the cats tolerate the procedure without sedation. The cat is placed in dorsal or lateral recumbency, depending on the operator's habits and the patient's condition. The abdominal hair is clipped and coupling gel is applied to the skin⁸⁻¹⁰.

B-mode ultrasonography is the procedure of choice to describe the shape, the size, the outline and the echogenicity of the adrenal glands. High frequency linear or microconvex transducers (>7,5 MHz) must be used given the small size of the organ⁸⁻¹⁰.

To visualize the left adrenal gland, the cat is placed in dorsal recumbency. The transducer is oriented in a sagittal plane. The left renal artery is followed

medially from the cranial pole of the kidney to the aorta. The adrenal gland is imaged cranio-ventral to the left renal artery and caudal to the origin of the cranial mesenteric artery, as shown in Figure 6⁹. The left adrenal gland can be more difficult to find in cats than in dogs because the left kidney is more mobile¹¹.

The cat can be placed in left lateral or dorsal recumbency to search for the right adrenal gland. The transducer is placed in a sagittal plane caudal to the rib cage. It is moved medially from the cranial pole of the right kidney to the caudal vena cava. The right adrenal gland is imaged slightly dorso-lateral to the caudal vena cava, caudal to the caudate process of the liver (Figure 7)⁹.

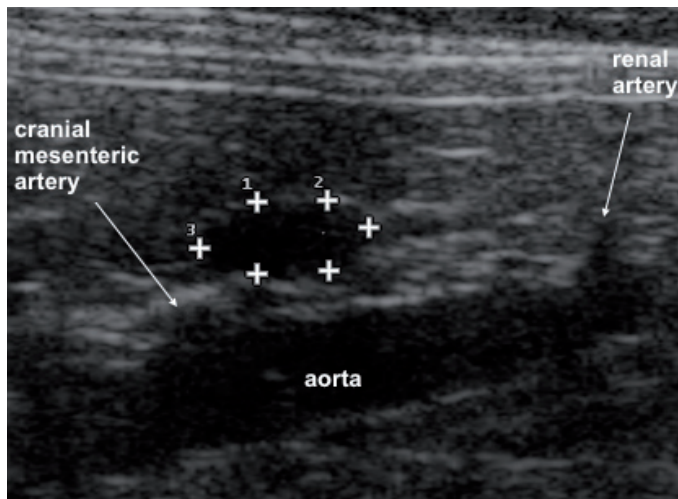


FIGURE 6 SAGITTAL IMAGE OF THE LEFT ADRENAL GLAND WITH VASCULAR LANDMARKS IN A NORMAL CAT. DISTANCE 1 AND 2 ARE CRANIAL AND CAUDAL HEIGHT RESPECTIVELY AND DISTANCE 3 IS THE LENGTH. CRANIAL IS TO THE LEFT.

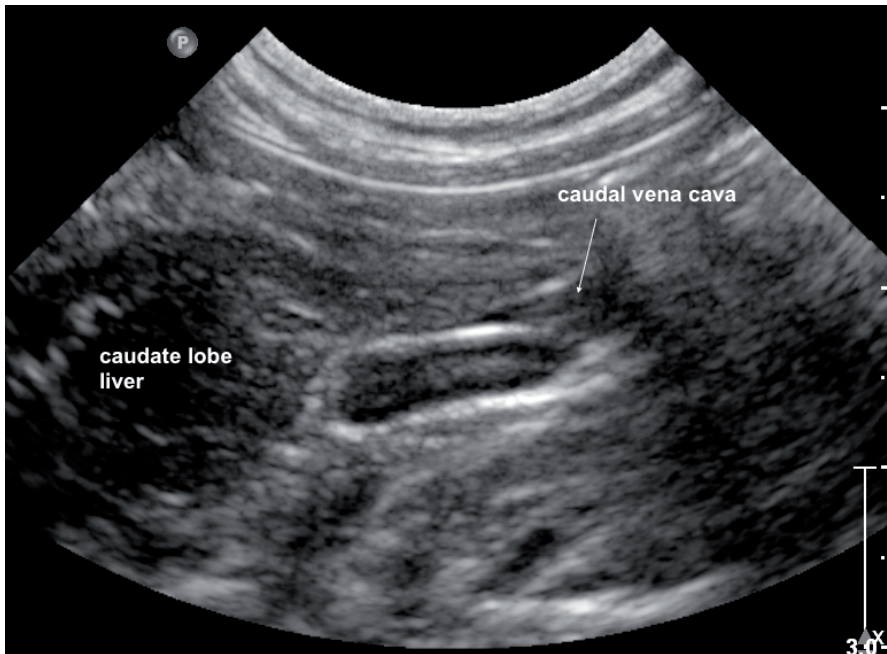


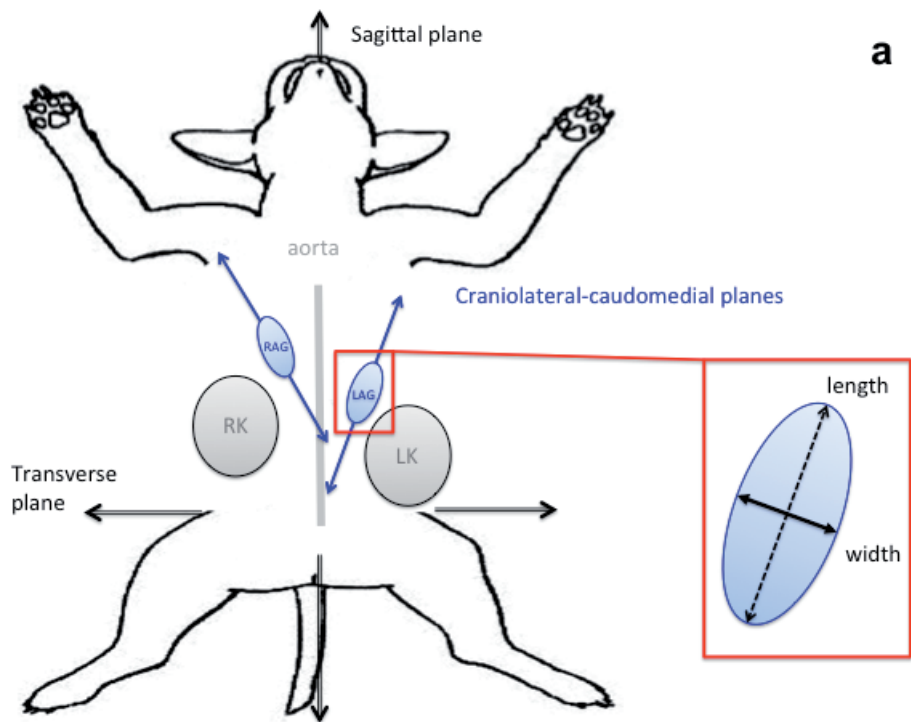
FIGURE 7 SAGITTAL ULTRASONOGRAPHIC IMAGE OF THE RIGHT ADRENAL GLAND WITH ANATOMICAL LANDMARKS IN A NORMAL CAT. CRANIAL IS TO THE LEFT.

As in dogs, both adrenal glands are not exactly in the sagittal plane of the cat's body. The cranial pole of the gland is mildly deviated laterally¹¹. Accordingly, the transducer should be rotated from the midline sagittal plane (assessed by a longitudinal scan of the aorta or the caudal vena cava) to a craniolateral-to-caudomedial oblique plane (Figure 8a)¹².

The identification of both adrenal glands can be confirmed by Doppler ultrasonography of the phrenico-abdominal vein which courses ventral to the mid body of the gland⁹. However the small vascularization within the adrenal gland cannot be assessed by Doppler ultrasonography.

The adrenal glands are mainly scanned in a sagittal plane to measure the maximal long and short axis. A transverse ultrasonography of the gland is also described but more difficult to obtain^{8,10}. Compared to post-mortem measurements, the short-axis measurement in a transverse plane was considered unreliable in cats⁸.

Although the long axis of the adrenal gland was widely considered as the length in the literature, the short axis appeared be called height, width, thickness or diameter without clear anatomical definition. The name of the adrenal measurements is defined in figure 8.



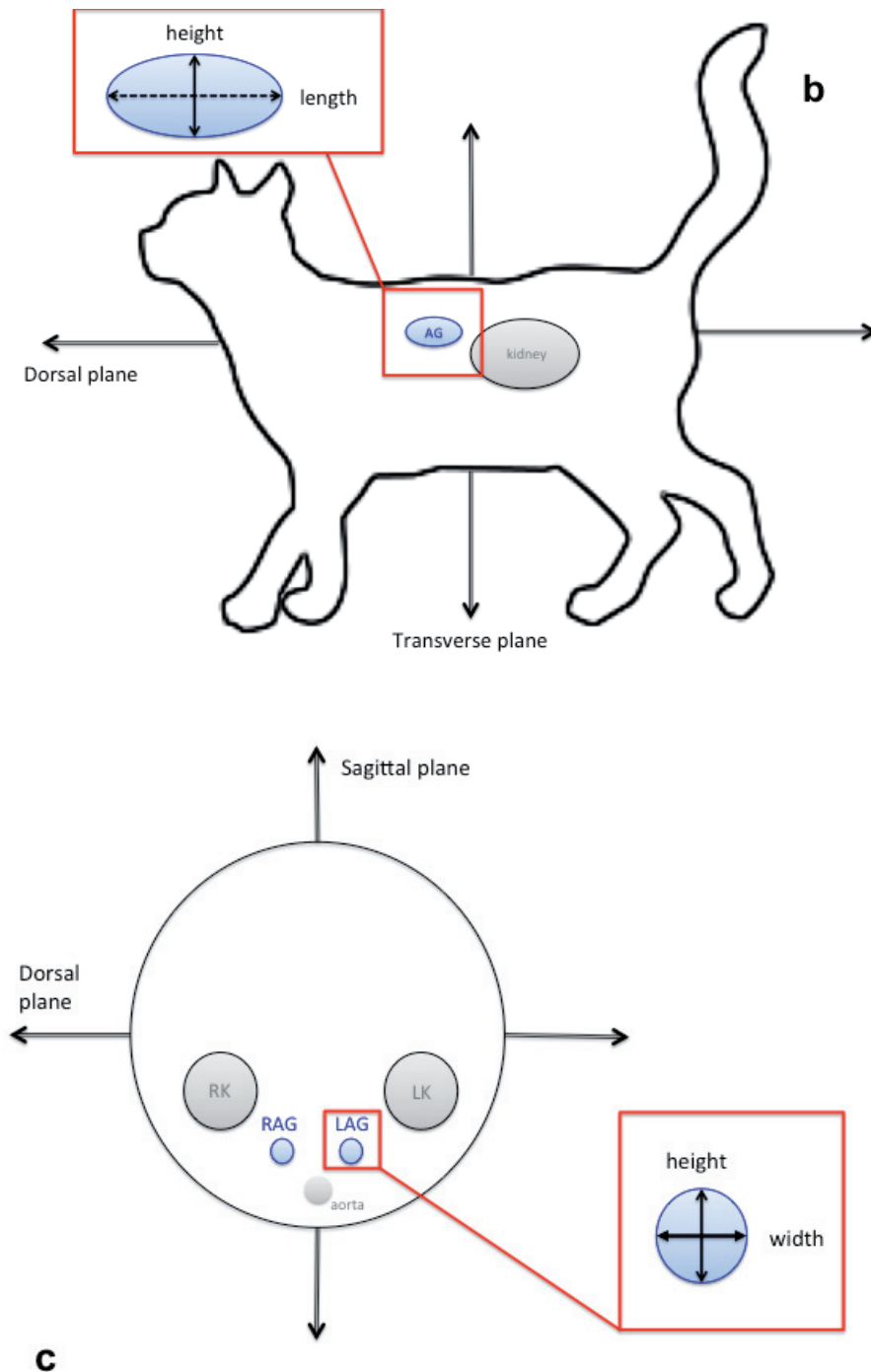


FIGURE 8 DEFINITION OF THE ULTRASONOGRAPHIC SCAN PLANES AND THE MEASUREMENTS OF THE ADRENAL GLANDS. (A) DORSAL PLANE. (B) SAGITTAL PLANE. (C) TRANSVERSE PLANE.

1.3 NORMAL ULTRASONOGRAPHIC APPEARANCE OF THE FELINE ADRENAL GLANDS

In contrast to the dog, the left and right adrenal glands have a similar shape in cats⁹. As shown in Figure 6, they are oblong, most often bilobed, hypoechoic and surrounded by a thin hyperechoic halo¹⁰. Occasionally, two concentric layers can be visualized with a more echoic centre and a hypoechoic periphery. It is not known whether these two layers represent the medulla and the cortex or varying amounts of fat deposition. The gland is surrounded by a hyperechoic halo of retroperitoneal fat, most likely masking the thin capsule⁹. Microscopic adrenal mineralization may be present in up to 30% of normal cats¹. It appears as a small hyperechoic focus with or without a distal acoustic shadow, in one or both adrenal glands⁹.

The normal ultrasonographic measurements of the adrenal glands are summarized in Table 1. The size of the adrenal glands is not correlated to bodyweight, body surface or body condition score¹⁰.

Ultrasonographic measurements in mm		Healthy cats (n=10)#	Healthy cats (n=20)§	Sick cats without endocrinopathies (n=24)@
		<i>mean ± SD</i>	<i>median (range)</i>	<i>mean ± SD</i> <i>median (range)</i>
Left adrenal gland	Length	10.7 ± 0.4	8.9 (4.5-13.3)	11.3 ± 2.8 10.6 (7.1-19.5)
	Height	4.3 ± 0.3	3.9 (3.0-5.3)	3.8 ± 0.8 3.6 (2.8-4.7)
Right adrenal gland	Length	10.7 ± 0.4	9.8 (6.7-13.7)	9.8 ± 2.4 9.9 (5.4-13.7)
	Height	4.3 ± 0.3	3.9 (2.9-4.5)	4.5 ± 1.0 4.2 (3.4-7.1)

TABLE 1 ULTRASONOGRAPHIC MEASUREMENTS OF THE ADRENAL GLANDS IN HEALTHY AND CHRONICALLY SICK CATS. # CARTEE ET AL., 1993, § ZIMMER ET AL., 2000, @ ZATELLI ET AL., 2007.

1.4 ADRENAL DISEASES AND REVIEW OF THE ULTRASONOGRAPHIC FINDINGS

1.4.1 HYPERADRENOCORTICISM (HAC)

Naturally occurring Cushing's syndrome is less common among cats as compared with dogs. Less than 100 feline cases are reported in literature²¹. In 80% of the cats, pituitary-dependent hyperadrenocorticism (PDH) is responsible for Cushing's syndrome. From these, more than 50% of the cats have a pituitary tumour that is large enough to be visualized with CT (19/31 reported cats) or MRI (3/5 reported cats) while in nearly a half of the PDH cats, the tumor is microscopic and cannot be seen^{6,13-15}. Most PDH are due to a pituitary adenoma, although a few cats with a pituitary carcinoma have been diagnosed (no ratio available)⁶. In the remaining 20% of the cats, an adrenal tumour-dependent hyperadrenocorticism (ATH) is the cause of Cushing's syndrome. Among ATH, 50% are adenomas and 50% are carcinomas⁶.

Hyperadrenocorticism is a disease of middle-aged and older cats. Cats diagnosed with PDH were 5 to 16 years (mean 10,7 years) and those with ATH were 8 to 15 years (mean 12 years). The main complaint was difficulty to regulate diabetes mellitus⁶.

Abdominal ultrasonography has been described as a discriminatory test for pituitary versus adrenal origin of feline hyperadrenocorticism. The US findings of fifty-one cats with HAC have been reported in multiple studies, including 40 cats with PDH and 11 cats with ATH (Table 2)^{6,16-26}.

	Ultrasonographic findings	Number of cats	Inconclusive US studies	Misleading US studies
Cats with ATH (11 cats)	<u>Unilateral mass, contralateral small</u>	4	1/11	2/11
	<u>Unilateral mass, contralateral not seen</u>	4		
	<i>Bilateral enlargement</i>	2		
	<i>Adrenal not seen</i>	1		
Cats with PDH (40 cats)	<u>Both adrenal normal</u>	3	3/40	5/40
	<u>Bilateral enlargement</u>	29		
	<i>One normal/ enlarged adrenal, contralateral not seen</i>	5		
	<i>Adrenal not seen</i>	3		
Total		51	4/51 (7,8%)	7/51 (13,7%)

TABLE 2 ABDOMINAL ULTRASOUND INTERPRETATIONS FROM 55 CATS WITH NATURALLY OCCURRING HYPERADRENOCORTICISM (ADAPTED FROM FELDMAN, SKELLY, NEIGER, SPADA, BROWN, CALSYN, SMITH, CROSS)

Cats with PDH showed symmetrical normal-to-enlarged adrenal glands while cats with ATH showed asymmetry of the adrenal glands, mainly illustrated as a unilateral adrenal mass with a smaller or not visualized contralateral adrenal gland. Using these criteria, 40/55 cats (80%) had a correct ultrasound diagnosis, 4/51 cats had inconclusive results (both adrenal gland not detected) and 7/51 cats had misleading results (cats with PDH with only one adrenal gland detected or cats with ATH with bilateral adrenomegaly) (Table 2). Although ultrasonography is an excellent tool for discriminating ATH from PDH, it remains complementary to the endocrine tests⁶.

CT and MRI are used for diagnostic imaging of the pituitary gland, as a diagnostic tool and for presurgical screening for PDH. Little information is available on CT and MRI examination of adrenal glands in feline patients.

1.4.2 SEX HORMONES-SECRETING NEOPLASIA (SHSN)

Adrenocortical tumours may excessively secrete one or multiple steroids, leading to hyperprogesteronism, hyperaldosteronism and/or hyperadrenocorticism⁶. As progesterone is also a precursor of other sex hormones, hyperprogesteronism may be associated with increased serum basal concentrations of various sex hormones, such as oestrogens and androgens⁵. Progesterone may simulate the actions of cortisol and have an intrinsic glucocorticoid effect^{5,6}.

Nine cats are reported in the literature with a diagnosis of sex hormones-secreting adrenocortical neoplasia^{5,6,27-33}. They were all neutered, 6 males and 3 females, with a mean age of 9,9 years (range: 7,0 to 15,0 years old). The clinical presentation was similar to hyperadrenocorticism such as unregulated diabetes mellitus, with some more specific clinical signs such as behavioural and genital changes in neutered cats.

In all cases, abnormal ultrasonographic findings were reported demonstrating the value of adrenal ultrasound as a screening test in cats suspected of adrenal disease^{5,6,27-33}. A unilateral adrenal mass was present in 7/9 cats^{5,6,27,28,30,31,33}. The contralateral adrenal gland was not visualized in the older reports (4 cats) but was described as small or normal in the most recent case reports. The adrenal lesions were characterized by a well-delineated, round, homogeneous or heterogeneous mass, sometimes creating a mass effect on the ipsilateral kidney. The diameter of the adrenal mass varied from 10 to 46 mm. Adrenocortical carcinoma was diagnosed in 4/7 cats and adrenocortical adenoma in one cat. One cat was diagnosed with an adrenocortical tumour without specific histopathological diagnosis and there was no histopathological

analysis in one cat. In 2/9 cats, the ultrasonographic findings were misleading. One cat was presented with bilateral moderate smooth adrenomegaly without histopathological analysis reported²⁹. Another cat with heterogeneous bilateral adrenal masses was diagnosed with bilateral adrenocortical carcinoma³².

All 9 cats showed high basal serum progesterone concentration and normal to low basal serum cortisol concentration. Two cats were reported with concurrent increased plasma aldosterone concentration^{5,30}. Intermediate hormones in the steroid biosynthesis pathways were variably tested. As reported in Table 3, an accurate diagnosis could be obtained through a combination of basal serum concentration testing of these hormones and ultrasonography³¹.

Cats	Endocrine blood tests	PG	17OHP	ASD	TESTO	PAC	ESTR	Ultrasound (adrenal)	Outcome	Histo-pathological
	CORT						ESTR			
Boord M. & Griffin C. 1999	=	↑	NE	NE	NE	NE	NE	Mass cranial to the left kidney	Adrenalectomy (alive after 12 months)	LAG adrenocortical carcinoma RAG not visualized
Rossmel JH et al. 2000	=	↑	NE	NE	↑	NE	=	RAG mass (27.4 mm) LAG not identified	Necropsy	RAG adrenocortical adenocarcinoma LAG not visualized
Feldman EC & Nelson RW. 2004	↓	↑	NE	NE	NE	NE	NE	Adrenal mass	Adrenalectomy (alive after 12 months)	Adrenocortical tumour
Boag AK et al. 2004	=	↑	=	NE	↑	NE	↑	Bilateral smooth adrenomegaly (RAG 12 mm, LAG 10 mm)	Euthanasia	NE
DeClue AE et al. 2005	=	↑	=	=	=	↑	=	RAG mass (25 mm)	Adrenalectomy (deceased after 4 days)	RAG adrenocortical carcinoma LAG grossly normal
Briscoe K et al. 2009	=	↑	NE	↓	NE	↑	NE	LAG mass (46 mm) Small RAG (1.8 mm)	Euthanasia	NE
Millard RP et al. 2009	= ↓ after ACTH stim	↑	↑	↑	↑	=	=	RAG mass (18 mm) Normal LAG (4 mm)	Adrenalectomy (alive after 8 weeks)	RAG adrenocortical adenoma LAG grossly normal
Quante S et al. 2009	↓	↑	=	NE	=	NE	NE	Bilateral masses (RAG 18 mm, LAG 29 mm)	Euthanasia	Bilateral adrenocortical carcinomas
Meler EN et al. 2011	= ↓ after ACTH stim	↑	↑	↑	NE	NE	↑	RAG mass (7 to 10 mm) Normal LAG (3.6 mm)	Adrenalectomy (unrelated euthanasia after 10 months)	RAG adreno-cortical carcinoma Small irregular LAG
Reference ranges	2-79 µg/L 30 & 60 min after 0.125 mg ACTH IM = 36-126 µg/L ¹⁹	<0.3 ng/mL ⁶	0.08-0.2 ng/mL ³³	1.4-3.2 ng/mL ³³	0.17-0.3 ng/mL ³³	4-618 pg/mL ⁹ 11-294 pg/mL ³³	56-74 pg/mL ³³	Normal adrenal thickness = 2.9-5.3 mm ⁹		

TABLE 3 BASAL SERUM CONCENTRATION OF INTERMEDIATE HORMONES FROM THE STEROIDS BIOSYNTHESIS PATHWAYS, ULTRASONOGRAPHIC AND PATHOLOGICAL FINDINGS IN THE NINE CATS REPORTED WITH SEX HORMONES-SECRETING NEOPLASIA^{5,6,27-33}. CORT: CORTISOL, PG: PROGESTERONE, 17OHP: 17 ALPHA-HYDROXYPROGESTERONE, ASD: ANDROSTENEDIONE, TESTO: TESTOSTERONE, PAC: ALDOSTERONE, ESTR: ESTRADIOL, ACTH: ADRENOCORTICOTROPHIC HORMONE, RAG: RIGHT ADRENAL GLAND, LAG: LEFT ADRENAL GLAND.

1.4.3 PRIMARY HYPERALDOSTERONISM (PHA)

Hyperaldosteronism is an abnormally increased secretion of mineralocorticoids such as aldosterone from the most external layer of the adrenal cortex, the zona glomerulosa.

Hyperaldosteronim secondary to stimulation of the renin-angiotensin system can be caused by kidney or heart failure for instance. Adrenal ultrasonography may be unremarkable in this case, although cardiac or renal lesions may be noticed ultrasonographically.

Primary hyperaldosteronism, also known as Conn's disease, is defined as the autonomous secretion of the hormone by abnormal cells within the adrenal cortex, leading to an excess of aldosterone associated with decreased renin concentration, hypokalaemia, potassium diuresis, metabolic alkalosis and systemic hypertension^{6,34,35}. Primary hyperaldosteronism can be concomitant with hyperadrenocorticism, hyperprogesteronism or multiple endocrine neoplasia^{5,25,30,36}.

Forty-two cats with hyperaldosteronism are reported in the literature from which 26 cases were confirmed by histological or cytological analysis^{5,25,30,36-45}. Eleven cats were diagnosed with an adrenocortical carcinoma (42,5%), 10 with an adrenocortical adenoma (38,5%), 3 with adrenal micronodular hyperplasia (11,5%) and 2 were unclassified (7,5%). The adrenal lesions were bilateral in 5 cats (19%) and unilateral in 21 cats (81%).

From the confirmed cases, the cats were geriatric (5 to 20 years old, mean 12 years old) and mostly neutered (19 neutered males, 7 neutered females). Breeds included a majority of Domestic shorthair, but Domestic longhair, British shorthair, Siamese, Burmese, Burmilla, Tonkinese and Persian were also represented^{25,30,36-45}. Two main syndromes, hypokalaemic polymyopathy and systemic hypertension, dominated the clinical presentation, although both were rarely present at the same time.

Ultrasonographic findings in primary hyperaldosteronism included adrenal enlargement, adrenal rounding, the presence of a hypoechoic or heterogeneous mass, hyperechoic spots with or without distal acoustic shadowing and invasion of the caudal vena cava³⁴. In the 3 cats with micronodular hyperplasia, ultrasound failed to detect an adrenal mass and the adrenal glands were described as “somewhat thickened” in 1 cat and normal in the others⁴².

Unilateral neoplasms are treated surgically whereas bilateral hyperplasia is managed medically. Ultrasound may be used to evaluate the subtype (hyperplasia, adenoma, adenocarcinoma) of hyperaldosteronism in cats, to choose between medical or surgical treatment³⁵.

1.4.4 IATROGENIC CUSHING’S SYNDROME

Iatrogenic Cushing’s syndrome is rare in cats, compared to humans and dogs and there is no report of the associated ultrasonographic findings in the current literature.

1.4.5 CONGENITAL ADRENAL HYPERPLASIA

Congenital adrenal hyperplasia refers to a group of autosomal recessive inherited diseases that result from a deficiency of one or several enzymes of the steroid biosynthesis pathways in the adrenal gland (Figure 3). Although it is the most common congenital endocrine disease in humans, congenital adrenal hyperplasia is very rarely reported in domestic animals. There are only two case reports in cats^{46,47}, both due to deficiency in 11 β -hydroxylase.

Affected cats are young neutered animals of undetermined sex (simultaneous male and female external genital organs) with polyuria and polydipsia and aggressive behaviour.

Ultrasonographic findings in these two cases were consistent with a normal adrenal thickness but an elongated shape^{46,47}. Only one adrenal measurement was reported (15,3 mm in length) in one case⁴⁶.

1.4.6 HYPOADRENOCORTICISM

Primary hypoadrenocorticism is a rare disease in cats. Naturally occurring disease is the most common condition but two cases of adrenal lymphoma, one case of traumatic destruction of the adrenal gland and a few iatrogenic secondary hypoadrenocorticism are also described⁴⁸.

Cats with hypoadrenocorticism are of all ages (1,5-14 years) and show similar clinical signs to dogs: lethargy, depression, anorexia, weight loss, depression, weakness, dehydration, and hypothermia⁴⁹.

There is no description of ultrasonographic findings in feline hypoadrenocorticism.

1.4.7 OTHER ADRENAL NEOPLASMS

Adrenal tumours are reported less frequently in cats than dogs. The frequency of feline adrenal masses reported in feline tumour surveys is approximately 0.03 to 0.2%. Reported adrenal neoplasia are cortical adenoma (69%), cortical carcinoma (23%), metastatic lesions (18%), pheochromocytoma (8%) and medullary paraganglionoma (1 case). The percentages are deducted from a retrospective search in the Veterinary Medical Database (VMDB, 1985-1996)⁵⁰. In a more recent post-mortem retrospective study, adrenal metastases represented 60% of all adrenal neoplasms diagnosed in cats in the institution during the same period (1983-2003). The primary tumours involved were most commonly lymphoma, and less commonly transitional cell carcinoma, melanoma, thyroid follicular cell carcinoma and fibrosarcoma. Adrenal metastases were diagnosed in 14.8% of cats with disseminated cancer⁵¹.

The main ultrasonographic finding was a unilateral adrenal mass, although a normal ultrasonography or bilateral masses were also described⁵²⁻⁵⁴. A bilateral adrenal tumour of different origin is reported in a cat with a left pheochromocytoma and a right secreting adrenocortical adenoma²². In a retrospective post-mortem study, half of the cats with adrenal metastases were

bilaterally affected and 60% were severely obliterating the gland. Unfortunately, there was no ultrasonographic description available in this study⁵¹.

1.5 MORPHOLOGICAL ADRENAL VARIATIONS ASSOCIATED WITH BIOCHEMICAL CHANGES IN SICK CATS

Stress and a variety of non-adrenal diseases are reported to affect the hypothalamic-pituitary-adrenocortical (HPA) axis and the sympathetic system of the adrenal medulla, in humans and numerous domestic or wild animal species. False positive test results (UCCR, LDDS test, ACTH stimulation test) are reported in sick dogs screened for hyperadrenocorticism⁵⁵. Adrenal gland or pituitary gland insufficiencies were reported in one study as common in dogs with acute critical illness⁵⁶. Hypercortisolemia and larger adrenal corticomedullary ratios are reported in male captive cheetahs compared to free-ranging cheetahs⁵⁷.

In cats, the adrenal function was tested in sick hospitalized cats, in diabetic cats, in hyperthyroid cats and in cats with pheochromocytoma compared to healthy cats. Results of an ACTH stimulation test and a low dose dexamethasone suppression test (LDDS) at 0.1 mg/kg were within normal limits in all except hyperthyroid cats⁵⁸⁻⁶⁰. Urine cortisol-to-creatinine ratio (UCCR) was higher in sick animals, in hyperthyroid cats and in healthy hospitalized animals⁶¹⁻⁶³. Plasma normetanephrine was also significantly higher in sick cats with non-adrenal disease compared to healthy cats, but still lower than in one cat with pheochromocytoma⁶⁴.

The biological changes in sick or hospitalized cats were not associated with ultrasonographic changes in the adrenal glands. Several studies concluded that cats with chronic non-adrenal disease or diabetic cats had normal-sized adrenal glands^{10,59}.

The biological results observed in hyperthyroid cats were associated with controversial ultrasonographic findings. One study did not find any clinically significant difference in adrenal size between healthy, chronically sick and hyperthyroid cats⁶¹. This is not in agreement with a histopathological study, showing that the adrenal glands of 8/23 hyperthyroid cats showed nodular hyperplasia of the zona glomerulosa and fasciculata⁶⁵.

Mild to moderate multifocal nodular adrenal hyperplasia is also described in some cats with acromegaly⁶⁶. The UCCR is also elevated in cats with acromegaly but the ACTH stimulation test and LDDS test results are normal. Bilateral adrenomegaly was detected ultrasonographically in 4/17 cats with acromegaly (24%). It is considered most likely in long standing acromegaly⁶⁷.

The effect of stress in cats with feline idiopathic cystitis (FIC) was studied as an underlying cause for the disease. Some studies focused on the functional and morphological characteristics of the adrenal glands in FIC cats. A lower response to ACTH stimulation was reported. Plasma catecholamine concentrations were higher in FIC cats compared to healthy cats. However the UCCR was not different between the two groups. Post-mortem mean weight and volume of the adrenal glands were significantly smaller in cats with FIC^{68,69}. There are no ultrasonographic data about cats with FIC, and it is not known whether this smaller size can be detected ultrasonographically.

1.6 CONCLUSION

Ultrasonography of the adrenal glands is the routine tool to visualize and assess adrenal-related diseases in cats. On the contrary, in human medicine, the gold standard in adrenal imaging is a combination of morphological and functional cross-sectional imaging (CT, MRI, Positron Emission Tomography, PET/CT and scintigraphy) to characterize more specifically an adrenal lesion and to differentiate benign from malignant lesions⁷⁰. Cross-sectional imaging of the feline adrenal glands is not yet reported in the veterinary literature.

Adrenal ultrasonography is mainly used in cats to detect adrenal asymmetry, which is suspicious for adrenal neoplasia (adrenal tumour-dependant hyperadrenocorticism, hyperaldosteronism, sex-hormone secreting neoplasia, pheochromocytoma, non-secreting neoplasia, metastasis). It is important to notice that adrenal ultrasonography is less reliable in case of bilateral hyperplasia (pituitary-dependant hyperadrenocorticism, acromegaly, hyperthyroidism, hyperaldosteronism, 11 β -hydroxylase deficiency). Ultrasound can be misleading in case of bilateral neoplastic infiltration (hyperaldosteronism, two concurrent neoplasms, metastasis). The ultrasonographic appearance is not pathognomonic for any disease and it remains complementary to endocrine biological testing to obtain a final diagnosis.

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2 SCIENTIFIC AIMS

Ultrasonographic examination of the feline adrenal gland has become a routine procedure in veterinary medicine due to improvement of the operator experience, knowledge of the US abdominal anatomy and improvement of ultrasound equipment. Additionally, compared to cross-sectional imaging modalities, such as computed tomography or magnetic resonance imaging, ultrasonography is more accessible, less expensive, does not require general anaesthesia and allows real-time procedures to be performed.

A few studies describe the normal ultrasonographic appearance of the feline adrenal glands. The ultrasonographic findings of some adrenal diseases, such as hyperaldosteronism and hyperadrenocorticism, have also been described in multiple case reports. However, as the pathological conditions of the feline adrenal glands are relatively sparse, original studies including a high number of cases are lacking.

The main objective of this thesis is to optimize the US examination procedure of the feline adrenal glands and, using this protocol, to describe the US appearance of the adrenal gland in normal cats (awake and anesthetized) and in sick cats.

The specific aims are:

- To standardize the interpretation of the ultrasonographic examination and the ultrasonographic measurements of the adrenal gland in normal cats (chapter 3).
- To apply this technique to healthy cats and cats with endocrine adrenal diseases (chapter 4).
- To study the effect of external factors, such as concurrent chronic diseases, stress or anaesthesia, on the ultrasonographic appearance of the adrenal glands in cats (chapter 4 to 6).

3 ULTRASONOGRAPHIC EXAMINATION OF THE FELINE ADRENAL GLANDS: INTRA- AND INTEROBSERVER VARIABILITY

Interpretation of ultrasonographic measurements requires an understanding of the source and the magnitude of variation. A substantial part of the variation can be accounted to the observer, the equipment or the animal¹. The aim of the study is to evaluate which adrenal gland measurement is the least variable within and between observers. Three experienced ultrasonographers examined 6 cats at 3 different times according to a strict scanning protocol. Seven ultrasonographic measurements were performed on each adrenal gland (maximal length on sagittal images, maximal height at the cranial and caudal poles on sagittal and transverse images, and maximal width of the cranial and caudal poles on transverse images). Height measurements in both planes showed the lowest variability within and between observers in comparison to length and width measurements. Descriptive ultrasonographic features such as echogenicity of the gland, presence of hyperechoic spots or layering assessment demonstrated a satisfactory to good intra- and interobserver agreement whereas the shape assessment showed a very poor interobserver agreement. The results of this study describe a reliable scanning protocol that can be the basis for future adrenal ultrasonographic examinations for cats suspected of adrenal disease (hyperaldosteronism, hyperadrenocorticism, sex-hormone-producing tumour for instance).

Adapted from:

Combes A, Stock E, Van der Vekens E, Duchateau L, Van Ryssen B, Saunders JH. **Ultrasonographic examination of feline adrenal glands: intra- and interobserver variability.** Accepted in *Journal of Feline Medicine and Surgery*.

3.1 INTRODUCTION

Ultrasonography of the adrenal glands is the most commonly used imaging modality in the diagnosis of adrenal diseases in cats. The most determinant findings in adrenal ultrasonography are adrenal asymmetry or adrenal mass. Although changes in echogenicity or shape of the gland are systematically examined, size remains a predominant criterion in the adrenal ultrasonography².

Only two studies examined the reliability of the ultrasonographic measurements of the feline adrenal glands in healthy cats^{3,4}. A first study compared ultrasonographic measurements of the adrenal glands in anesthetized cats with post-mortem gross measurements. They concluded that ultrasonographic measurements underestimated the anatomic measurements (2 mm smaller). In the same study, the length and height were more reliable than the width³.

In a second study about reliability of the ultrasonographic measurements of the feline adrenal glands, they reported a 10-times serial examination on 3 cats. The coefficient of variation of ultrasonographic measurement of the adrenal glands was the lowest for the short-axis measurement (4.4-7.2 %). The maximal measurement variation in a day-to-day serial examination was 1.2 mm on 10 cats⁴.

Little is known about the within and between observers variability of ultrasonographic measurements of adrenal glands in cats. A study about intra- and interobserver variability of ultrasonographic measurements of the adrenal glands in dogs showed that the height of the caudal pole of both adrenal glands measured on sagittal images had the lowest intra- and interobserver variability⁵.

The aim of the study is to evaluate which adrenal gland measurement is the least variable within and between observers. This may help to determine if we

can assess more reliable normal and pathological threshold for the size of the feline adrenal glands.

3.2 MATERIALS AND METHODS

Six private-owned cats were involved, belonging to staff of the small animal clinic of the Veterinary Faculty of Ghent University. There were 2 castrated males and 4 neutered females. They ranged from 3 to 9 years (mean 5,7 years) and weighted 3 to 5 kg (mean 4,2 kg). The cats were clinically healthy, exempt of any disease in the past and at least until 6 months after the study.

The ultrasonographic examination was done without sedation, in dorsal recumbency with minimal manual restraint. The abdominal hairs were clipped and coupling gel was applied. The cats were scanned with a multifrequency (5-8 MHz) microconvex probe set on 8 MHz.

Three ultrasonographers were involved in the study, two ECVDI-diplomate (JHS, EV) and one last-year resident (AC). They operated with the same machine settings. At three different times the same day (> 1 hour apart), they were asked to scan both adrenal glands of each cat according to previously reported technique⁴. The operators could not be completely blinded as the cats were visible during the procedure, although the cats were scanned in a random and variable order at each of the three times.

The shape (bilobed, fusiform or rounded), the echogenicity (hypo-, iso-, hyperechoic compared to the surrounding fat) and the presence of layers or hyperechoic spots, with or without distal acoustic shadowing were recorded. According to a previously described protocol with strict scanning planes⁵, seven distances were measured (Figure 9 to 12):

- the maximal length (cranio-caudal) in sagittal plane,
- the maximal width (medio-lateral) of the cranial and the caudal poles in transverse plane,

- the maximal height (ventro-dorsal) of the cranial and caudal poles in sagittal and transverse planes.

The difficulty to find the adrenal gland and the behaviour of the cat were recorded. The ultrasound sessions were prepared by an additional external person (ES) to keep operators blindness about the cats they were scanning.

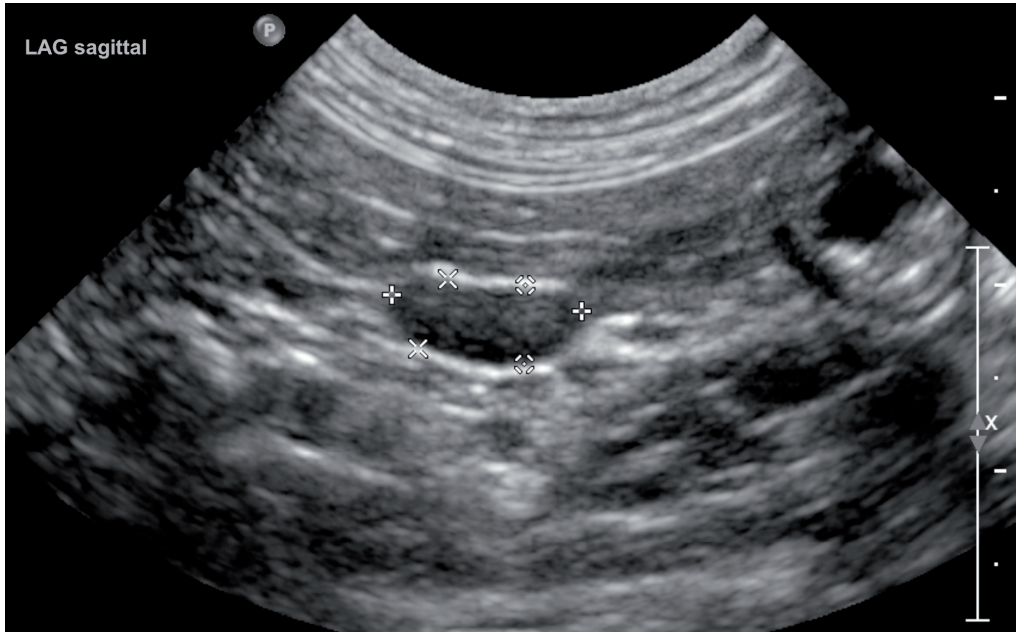


FIGURE 9 LENGTH (+) AND HEIGHT (x, ⊙) ULTRASONOGRAPHIC MEASUREMENTS OF THE LEFT ADRENAL GLAND IN A SAGITTAL PLANE. CRANIAL IS TO THE LEFT.

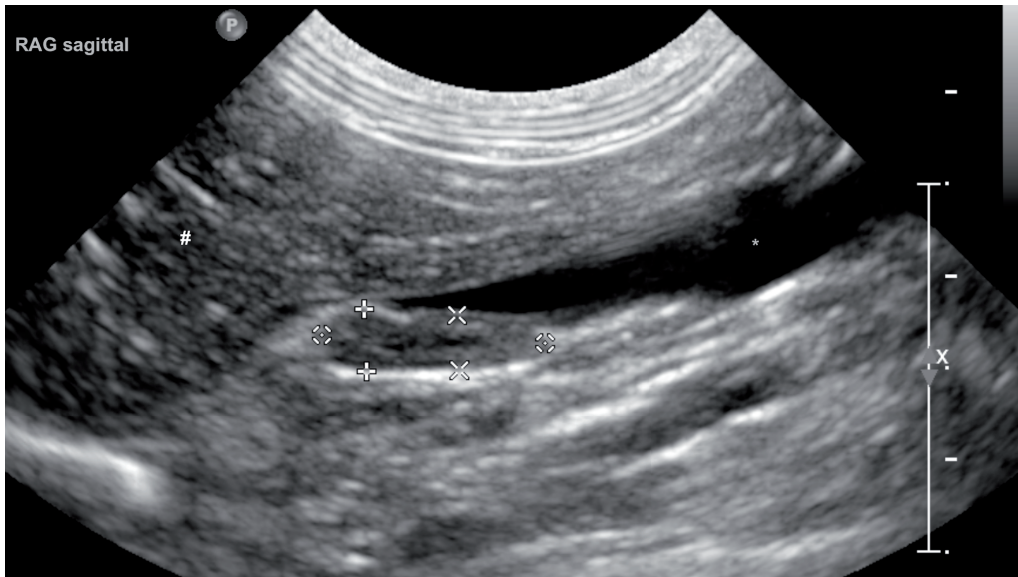


FIGURE 10 LENGTH (⊙) AND HEIGHT (+, x) ULTRASONOGRAPHIC MEASUREMENTS OF THE RIGHT ADRENAL GLAND IN A SAGITTAL PLANE. # LIVER, * CAUDAL VENA CAVA. CRANIAL IS TO THE LEFT.

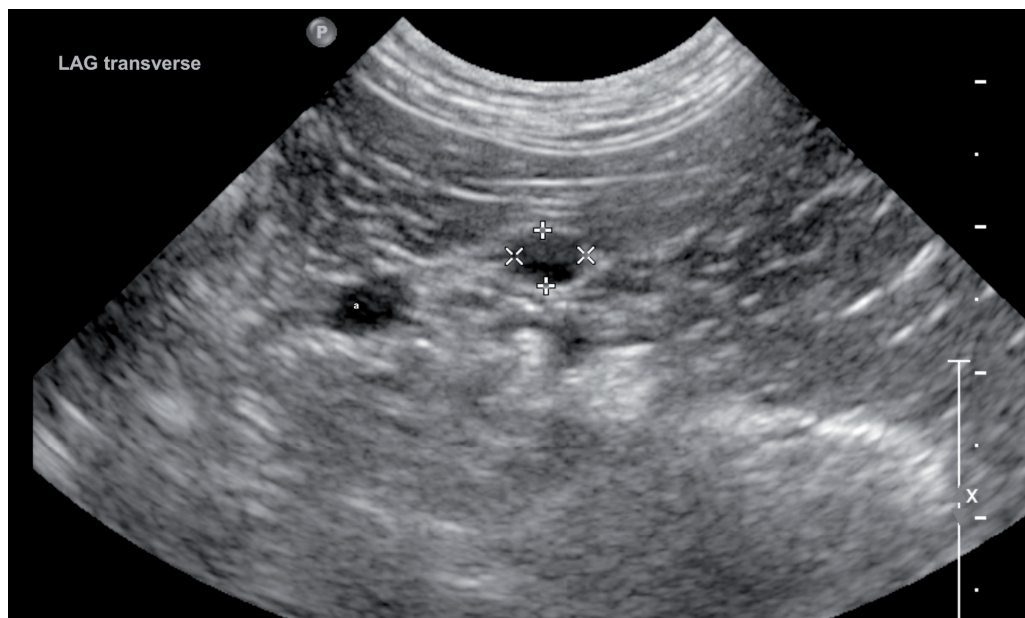


FIGURE 11 HEIGHT (+) AND WIDTH (x) ULTRASONOGRAPHIC MEASUREMENTS OF THE LEFT ADRENAL GLAND IN A TRANSVERSE PLANE. a AORTA. MEDIAL IS TO THE LEFT.

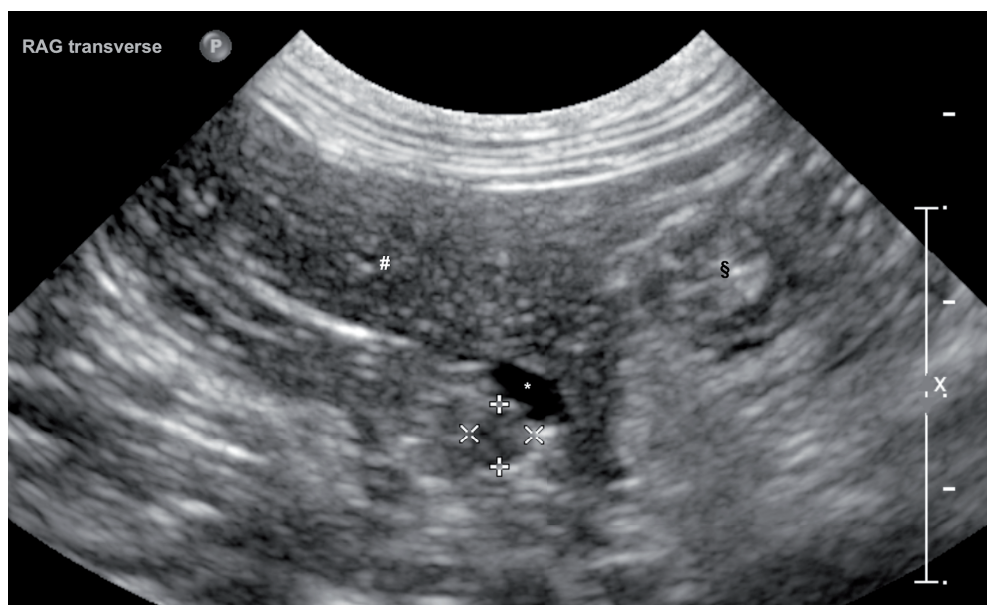


FIGURE 12 HEIGHT (+) AND WIDTH (x) ULTRASONOGRAPHIC MEASUREMENTS OF THE RIGHT ADRENAL GLAND IN A TRANSVERSE PLANE. # LIVER, * CAUDAL VENA CAVA, § DUODENUM. LATERAL IS TO THE LEFT.

A random effects model was used with cat, observer and measurement within observer as random effects⁶. Within the framework of the random effect model, three variance components were estimated, i.e., the variation within observers, σ^2_a , the variation due to observers, σ^2_i , and the variation due to cats, σ^2_c using restricted maximum likelihood techniques⁷. Based on these variance component estimates, limits of agreement (LA) were derived that contain 95% of the measurements of the same cat by the same observer (within observer LA), 95% of the measurements of the same cat but assessed by different observers (between observer LA) and 95% of the measurements of different cats assessed by different observers (between cats LA). Assuming normally distributed measurements, the limits of agreement were obtained from the observed average value for the particular measurement, together with the appropriate sources of variation, i.e., the variance components.

The cat behaviour and the difficulty to find the adrenal gland were considered as covariates and the effect of these covariates on the within observer variance of the adrenal measurements was evaluated with a paired Student's t-test, using cat and observer as a blocking factor.

For the descriptive characteristics (shape, echogenicity, hyperechoic spots, layering), the percentage of agreement both within and between observers was calculated and compared to the expected value under the hypothesis that the assessment was haphazard. Considering the shape or the echogenicity or the layering of the gland with 3 possible values, the expected value for pairwise agreement equalled 33% both within and between observers. Considering the presence or absence of hyperechoic spots, the expected value for pairwise agreement equalled 50% both within and between observers.

3.3 RESULTS

The estimated overall means (with 95% confidence intervals) of the adrenal gland measurements are presented in Table 4.

Adrenal measurements (mm)		Right adrenal gland		Left adrenal gland	
		<i>Mean</i>	<i>95% CI</i>	<i>Mean</i>	<i>95% CI</i>
Sagittal plane	Length	11.1	9.8-12.5	10.8	9.1-12.4
	Cranial height	3.6	3.3-4.0	3.3	2.8-3.9
	Caudal height	3.0	2.7-3.3	3.3	2.7-3.9
Transverse plane	Cranial height	3.6	3.3-3.9	3.4	2.8-4.0
	Cranial width	4.3	3.7-5.0	4.6	4.0-5.1
	Caudal height	3.3	2.7-3.8	3.4	2.8-3.9
	Caudal width	3.6	3.1-4.1	4.2	3.6-4.8

TABLE 4 ESTIMATED OVERALL MEANS WITH 95% CONFIDENCE INTERVALS (CI) OF THE ADRENAL MEASUREMENTS IN THE 6 CATS WITH THE 3 OBSERVERS.

The variance components are shown in Table 5 and the limits of agreement (LA), as defined earlier, in Figures 13 and 14. For all measurements, the extra variation between observers is moderate compared to the variation within observers and the extra variation due to cat.

As expected, length has the highest variability for both adrenal glands, with larger variability on the left side. The within observer LA equals (9.3;13.0 mm) for the right adrenal gland and (8.3;13.1 mm) for the left adrenal gland. The between observer LA equals (9.1;13.1 mm) for the right adrenal gland and (7.9;13.6 mm) for the left adrenal gland, only slightly broader than the within observer LA. The between cat LA, however, is substantially broader, and equal

to (7.9;14.4 mm) for the right adrenal gland and (6.9;14.6 mm) for the left adrenal gland.

The variance components of most of the short axis measurements are moderately larger in the right adrenal gland compared to the left one (Table 5).

			Variance components		
			Due to cat (σ^2_c)	Due to observer (σ^2_i)	Within observer (σ^2_a)
Right adrenal gland	Sagittal plane	Length	2.4406	0.1595	1.3209
		Cranial height	0.1036	0.0382	0.2826
		Caudal height	0.1576	0	0.1683
	Transverse plane	Cranial height	0.1613	0	0.2537
		Cranial width	0.3130	0.1204	0.8930
		Caudal height	0.2795	0.0558	0.2473
		Caudal width	0.2868	0.0122	0.6483
Left adrenal gland	Sagittal plane	Length	2.4901	0.7656	2.1977
		Cranial height	0.4344	0	0.1673
		Caudal height	0.4265	0.0371	0.1388
	Transverse plane	Cranial height	0.5413	0.0667	0.1638
		Cranial width	0.2153	0.1433	0.5005
		Caudal height	0.3988	0.0172	0.1342
		Caudal width	0.1637	0.1411	0.4371

TABLE 5 VARIANCE COMPONENTS OF THE ULTRASONOGRAPHIC ADRENAL MEASUREMENTS IN 6 CATS WITH 3 OBSERVERS.

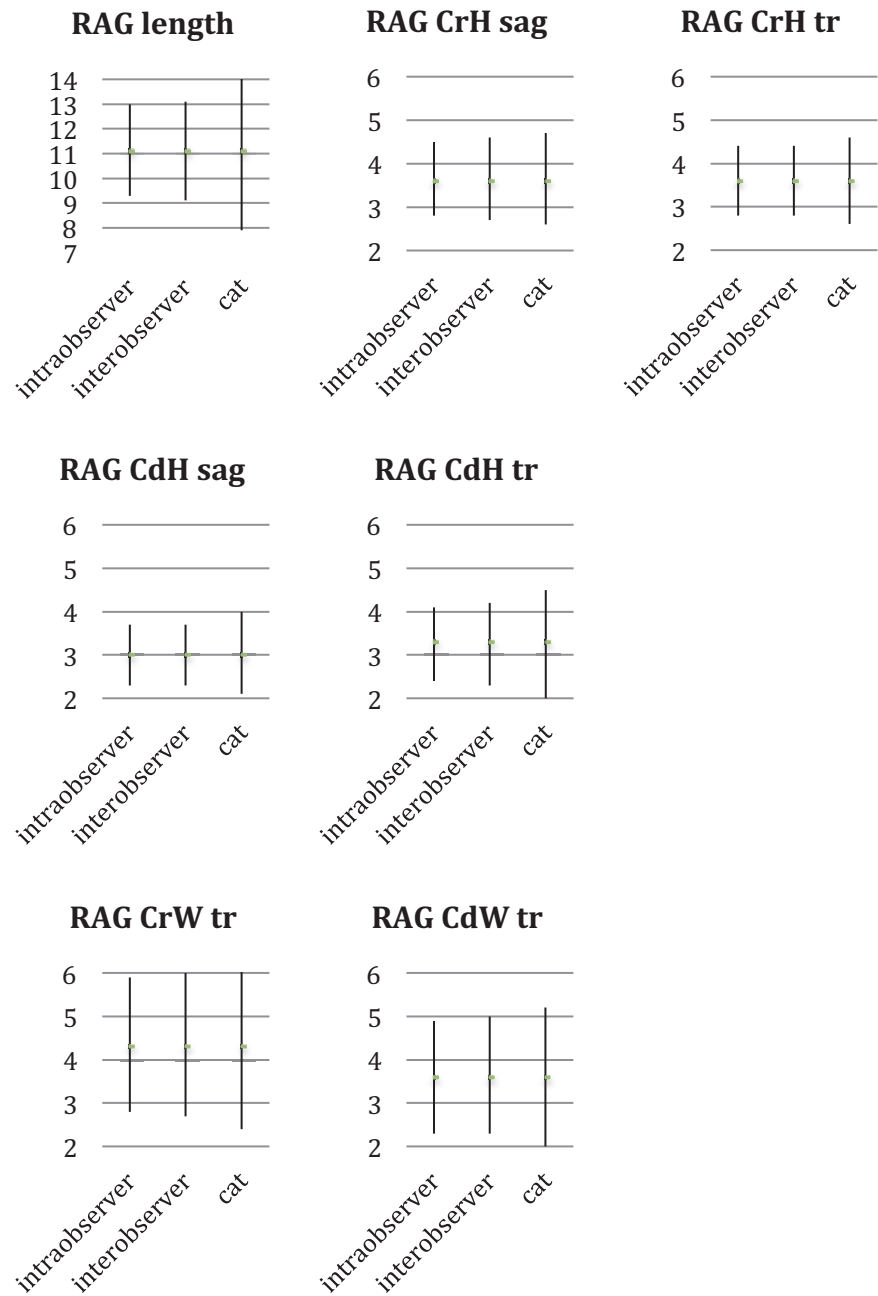


FIGURE 13 LIMITS OF AGREEMENT (MEAN AND 95% MEASUREMENTS) FOR RAG MEASUREMENTS WITHIN OBSERVERS (INTRAOBSERVER), MEASUREMENTS BETWEEN OBSERVERS (INTEROBSERVER) BOTH ON THE SAME CAT, AND MEASUREMENTS BETWEEN OBSERVERS ON DIFFERENT CATS (CATS). RAG RIGHT ADRENAL GLAND, CrH CRANIAL HEIGHT, CdH CAUDAL HEIGHT, CrW CRANIAL WIDTH, CdW CAUDAL WIDTH, SAG SAGITTAL PLANE, TR TRANSVERSE PLANE.

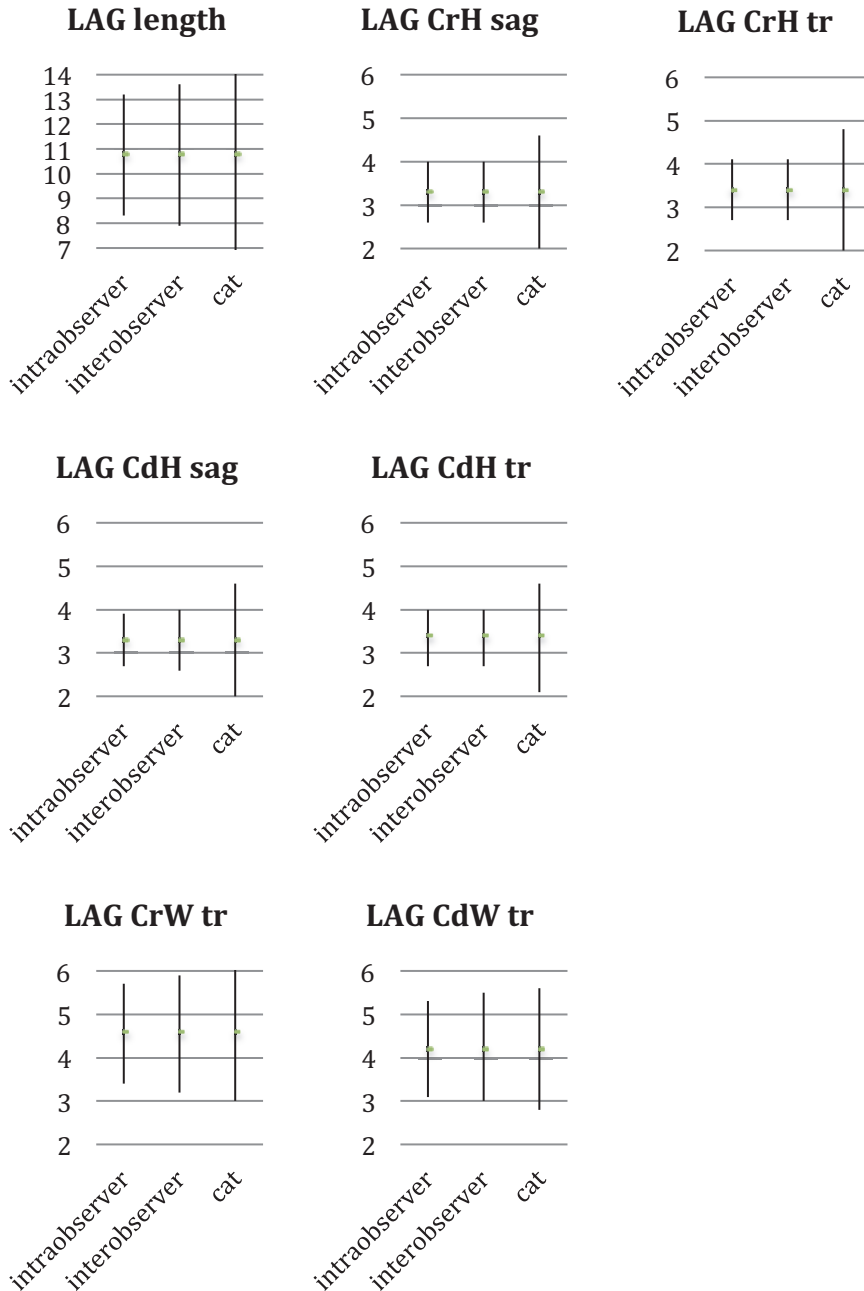


FIGURE 14 LIMITS OF AGREEMENT (MEAN AND 95% MEASUREMENTS) FOR LAG MEASUREMENTS WITHIN OBSERVERS (INTRAOBSERVER), MEASUREMENTS BETWEEN OBSERVERS (INTEROBSERVER) BOTH ON THE SAME CAT, AND MEASUREMENTS BETWEEN OBSERVERS ON DIFFERENT CATS (CATS). LAG LEFT ADRENAL GLAND, CrH CRANIAL HEIGHT, CdH CAUDAL HEIGHT, CrW CRANIAL WIDTH, CdW CAUDAL WIDTH, SAG SAGITTAL PLANE, TR TRANSVERSE PLANE.

The within and between observer variance components of the cranial height measurements are comparable if taken in a sagittal or a transverse plane. In the sagittal plane, the within observer LA equals (2.8;4.5 mm) for the right adrenal gland and (2.7-4.0 mm) for the left adrenal gland, whereas the between observer LA equals (2.7;4.6 mm) for the right and (2.6;4.0 mm) for the left adrenal gland, only slightly broader than the within observer LA. The between cat LA is only slightly broader, and equal to (2.6;4.7 mm) for the right adrenal gland and (2.0;4.6 mm) for the left adrenal gland. In the right adrenal gland, the within observer LA is substantially broader for the cranial height measurements compared to the caudal height measurements, in both scanning planes.

The within and between observer LA of the caudal height measurements are comparable if taken in a sagittal or a transverse plane. In the sagittal plane, the within observer LA equals (2.3;3.7 mm) for the right adrenal gland and (2.7-3.9 mm) for the left adrenal gland, whereas the between observer LA is the same, i.e., (2.7;3.7 mm) for the right and only slightly broader, i.e., (2.6;4.0 mm) for the left adrenal gland. A moderate increase was observed for the between cat LA, with values equal to (2.1;4.0 mm) for the right adrenal gland and (2.0;4.6 mm) for the left adrenal gland.

The within and between variance components are in most cases smaller for the height measurements than for the width measurements, except for the caudal width of the right adrenal gland in a transverse plane. On the contrary, the between cats variance component is similar between height and width measurements.

The within observer LA for cranial/caudal width measurements corresponds to (2.8;5.9 mm)/(2.3;4.9 mm) for the right adrenal gland and to (3.4;5.7 mm)/(3.1;5.3 mm) for the left adrenal gland, whereas the between observer LA for cranial/caudal width measurements corresponds to (2.7;6.0 mm)/(2.3;5.0 mm) for the right and (3.2;5.9 mm)/(3.0;5.5 mm) for the left adrenal gland. The

between cat LA to (2.4;6.2 mm)/(2.0;5.2 mm) for the right and (3.0;6.1 mm)/(2.8-5.6 mm) for the left adrenal gland.

The percentage of agreement both within and between observers about the descriptive ultrasonographic features of the adrenal glands are shown in Table 6.

	Intra-observer	Inter-observer
Shape	79%	31%
Echogenicity	93%	74%
Layering pattern	93%	89%
Hyperechoic spots	81%	78%

TABLE 6 PERCENTAGE OF AGREEMENT WITHIN AND BETWEEN OBSERVERS FOR DESCRIPTIVE ULTRASONOGRAPHIC FEATURES OF THE ADRENAL GLANDS IN CATS. THE EXPECTED VALUE UNDER THE HYPOTHESIS OF RANDOM CHOICE EQUALS 33% FOR THE THREE CATEGORY VARIABLES SHAPE, ECHOGENICITY AN LAYERING PATTERN, AND 50% FOR THE BINARY VARIABLE HYPERECHOIC SPOTS.

The echogenicity and layering description demonstrate a very good within observer agreement (93%). The shape description and the presence of focal spots demonstrate a moderate within observer agreement (80 and 81%). The between observer agreement on the shape of the adrenal gland is very poor (31%), even below the expected 33% if choices were made haphazard, whereas the between observer agreement for the presence of focal spots, the layering and the echogenicity is satisfactory (78%, 89% and 74%).

3.4 DISCUSSION

The agreement for the ultrasonographic adrenal measurements in healthy cats is overall high, both within and between observers. This result confirms the easiness of this procedure. A previous study describes the repeatability of the ultrasonographic adrenal measurements in cats the same day 10 times in

succession and twice on consecutive days. They found a small coefficient of variation on consecutive measurements (2.5-13.5% for the length and 4.4-7.2% for the short axis measurement) and the difference in adrenal measurements between the 2 days ranged from 0.3 to 1.2 mm. However, the latter study does not report the effect of multiple observers⁴.

In our study, the between observer limit of agreement is typically larger than the within observer limit of agreement. However, it is only very slightly larger in most cases. The good agreement between observers in the present study is interesting in a clinical situation, as different observers can reproduce the procedure without significant variation. It is hypothesized that a standardized protocol of ultrasonographic scanning and measurements is mandatory to keep the variation as low as possible.

The lack of a standardized scanning protocol in the previous studies about ultrasonographic adrenal measurements makes the comparison difficult between them and with our study^{3,4}. Although the length is clearly anatomically defined, the short axis measurements are more variably defined when called thickness, height or width. We constrain ourselves to strict scanning planes to define the height of the adrenal gland in a dorsoventral plane and the width in a mediolateral plane. In a previous study, according to their animal positioning, the maximum right adrenal width measured in left lateral recumbency may correspond to the width in the present study while the maximum left adrenal width measured in dorsal recumbency may correspond to the height⁴. In the present study, the width measurements are presenting much higher within and between observer variability compared to the height measurements. A previous study reports that the only significant differences between the post-mortem physical measurements and the ultrasonographic measurements are in width. The protocol appears comparable with the present study with length, thickness and width measurements³. Comparing the maximum short axis measurements reported as 5.3 mm in previous literature⁴, the present height

measurements never reach 5 mm whereas the width measurements reach 6 mm. The ultrasonographic adrenal width appears a less reliable measurement in cats. This result is likely due to the ill-defined medial and lateral borders of the glands compared to the dorsal and ventral outline, and the close contact with vascular landmarks (aorta and caudal vena cava for left and right adrenal glands respectively).

Another hypothesis to explain the increased variability of the width measurement may be the use of a transverse scanning plane to measure the width. If the scanning plane is oblique, there is a risk to overestimate the true width of the gland. This finding is reported in similar studies about ultrasonography of the canine adrenal glands and thyroid glands^{5,8}. It appears more difficult to obtain a true transverse plane of the gland as, in cats like in dogs, the adrenal gland lies not exactly in the sagittal plane of the body but slightly angled with a more laterally deviated cranial pole⁶. On the contrary, it is easier to obtain a true sagittal plane by aiming the maximum length of the gland. Regarding the height measurements, the within and between observer variability are similar if measured in sagittal or transverse planes. Indeed, if the adrenal gland is parallel to the surface of the transducer, when the scanning orientation changes from a sagittal to a transverse plane, the height of the gland remains perpendicular to the transducer, thus quite constant, whereas the width depends on the degree of rotation of the transducer and is therefore probably more variable. Briefly, a sagittal image of the adrenal gland is advised to measure the length and the height, starting from the longitudinal image of the aorta and aiming craniolateral-caudomedially to enlarge the adrenal gland to its maximal length and height and keeping it parallel to the surface of the transducer.

The within and between limits of agreement of the length measurements are wider for the left adrenal gland compared to the right one. The greater mobility of the left vascular and renal landmarks may account for the more difficult

sagittal scanning of the left adrenal gland and then a less reliable length measurement. On the contrary, the within and between limits of agreement of the height and width measurements are smaller for the left adrenal gland compared to the right one. Additionally, in the right adrenal gland, the cranial height measurement shows broader within observer limits of agreement than the caudal height measurement in any plane. These findings are similar to what is described in dogs and most likely due to the similar echogenicity and the close contact of the right adrenal gland, particularly the cranial pole, with the caudate lobe of the liver and the caudal vena cava⁵.

The agreement on the descriptive ultrasonographic features of the feline adrenal gland is satisfactory to very good, except for the assessment of the shape between observers. Despite a consensual scanning protocol, the descriptive features are very subjective. To reduce variability, the internal echogenicity and echostructure of the adrenal gland may be compared with adjacent landmarks such as aorta or caudal vena cava or adjacent organs such as liver or kidneys. However, the changes in shape of the gland are minor and too subjective to be evaluated reliably with ultrasound.

3.5 CONCLUSION

The within and between limits of agreement of the ultrasonographic adrenal measurements in healthy cats are overall narrow. This confirms the reliability of the ultrasonographic evaluation of the adrenal size in cats. Involvement of multiple ultrasonographers in the serial evaluation of an individual patient would most likely not change the ultrasonographic measurements of the adrenal glands in a clinically significant way. The main precautions to actually reduce the variation are: using a strict consensual scanning protocol and the same updated and calibrated ultrasound device. Considering the scanning protocol, height measurements in sagittal or transverse planes are more reliable than the length or the width measurements in transverse plane.

3.6 ACKNOWLEDGEMENTS

The authors would like to acknowledge Laure Gatel, Eva Vandermeulen and Miguel Campos for their contribution in the experiment.

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4 ULTRASONOGRAPHIC APPEARANCE OF ADRENAL GLANDS IN HEALTHY AND SICK CATS

The first part of the study aimed to describe prospectively the ultrasonographic features of the adrenal glands in 94 healthy cats and 51 chronically sick cats. This study confirmed the ultrasonography of adrenal glands in healthy and chronically sick cats is feasible. No statistically significant differences in ultrasonographic features between healthy and chronically sick cats were detected. The typical hypoechoic appearance of the gland surrounded by hyperechoic fat made it easily recognizable. A sagittal plane of the gland, not in line with the aorta, may be necessary to obtain the largest adrenal measurements. The reference intervals of adrenal measurements were inferred from the values obtained in the healthy and chronically sick cats (mean \pm 1.96*SD): the adrenal length was 6.9 to 14.4 mm; the cranial height was 2.0 to 5.7 mm; the caudal height was 2.2 to 5.2 mm. The second part of the study consisted in a retrospective analysis of the ultrasonographic examination of the adrenal glands in cats with adrenal diseases (6 hyperaldosteronism, 4 pituitary-dependent hyperadrenocorticism) and a descriptive comparison with the reference features obtained in the control groups from the prospective study. In all cats with hyperaldosteronism a unilateral severely enlarged adrenal gland was observed. However, a normal contralateral gland on ultrasonography did not preclude a contralateral infiltration of benign or malignant adrenal neoplasms. Moreover, benign and malignant lesions could not be differentiated based upon the ultrasonographic appearance of the adrenal gland. The ultrasonographic appearance of the adrenal gland in pituitary-dependent hyperadrenocorticism was a symmetrical adrenal enlargement, however 2/4 cases were within the reference intervals of adrenal size.

Adapted from: Combes A, Pey P, Paepe D, Rosenberg D, Daminet S, Putcuypys I, Bedu AS, Duchateau L, de Fornel-Thibaud P, Benchekroun G, Saunders JH. **Ultrasonographic appearance of adrenal glands in healthy and sick cats.** *J Feline Med Surg* 2013; 15(6): 445-57.

4.1 INTRODUCTION

Adrenal glands are currently assessed by ultrasonography in feline medicine based on two studies about healthy cats¹⁻². As non-adrenal diseases can modify the results of biological tests (UCCR, LDDS, ACTH stimulation), it can be hypothesized that they may impact on the ultrasonographic features of the adrenal glands. One study examined the ultrasonographic measurements of feline adrenal glands in sick cats without endocrine diseases³. The authors did not find any statistically significant difference compared with the two studies about healthy cats. The sick cats only presented a wider range of values compared to healthy cats. During the 14 years between the first and the last studies about ultrasonography of the feline adrenal glands, there was a noticeable improvement in ultrasonographic equipment and in scanning procedure and accuracy, which makes the comparison poorly reliable. The additional descriptions of changes in adrenal gland size in specific feline diseases are variable: significantly smaller adrenal glands were described in cats with idiopathic cystitis and adrenal glands were not enlarged in cats with diabetes mellitus⁴⁻⁵. Studies on the effect of hyperthyroidism on adrenal gland measurements show conflicting results⁶⁻⁷.

Variation in ultrasonographic features and size of adrenal glands in non-adrenal diseases could impact the specificity of ultrasonographic examination of adrenal gland for detection of adrenal gland hyperplasia or neoplasia, associated with hyperaldosteronism or hyperadrenocorticism and for the diagnosis of hypoadrenocorticism, sex hormone-producing tumour, pheochromocytoma or adrenal metastasis⁸. Moreover, there is no imaging study describing the ultrasonographic appearance of the adrenal glands in cats with adrenal gland diseases, for instance in hyperaldosteronism or hyperadrenocorticism. There are only multiple case reports and series describing the imaging findings with different precision levels (absent or subjective size evaluation up to 3-dimension measurements, vague up to very

descriptive ultrasonographic features like shape, contour, echogenicity, homogeneity).

The first part of the present report is a prospective study to assess adrenal gland features for healthy but also for cats with chronic non-adrenal diseases, with a standard ultrasonographic protocol using high-resolution ultrasound. We aimed to describe more accurately the relative difference in adrenal size, shape and echogenicity that may appear during a non-adrenal disease.

The second part of this report is a retrospective study about ultrasonographic findings in cats diagnosed with hyperaldosteronism, hyperadrenocorticism in two referral centres. The aim is to compare these findings with the findings from the control population recruited in the prospective study.

4.2 MATERIAL AND METHODS

4.2.1 PROSPECTIVE STUDY: CHRONICALLY SICK AND HEALTHY CATS

The chronically sick cats included in the study were evaluated at the small animal clinic of the Veterinary Faculty of Ghent University and the CHUVA of the Veterinary school of Alfort from January to December 2010. Fifty-one cats with clinical signs for more than 10 days that had an abdominal ultrasonography and a precise definite diagnosis of non-endocrine disease were included. Table 7 summarizes the different types of diseases encountered in this group. Cats with suspected or confirmed feline idiopathic cystitis, hyperadrenocorticism, hypoadrenocorticism, diabetes mellitus, hyperthyroidism, primary hyperaldosteronism, hypersomatotropism, aberrant production of sexual hormones were excluded. Mean duration of clinical signs was 112 days (SD = 116 days) with a range of 10 to 360 days (median = 90 days).

Diagnosis		Number of cats
<i>Degenerative disorders</i>	Chronic kidney disease (CKD)	10 including one with megacolon and one positive for Feline Leukaemia Virus
<i>Inflammatory disorders</i>	Parodontal disease	1
	Chronic allergic (gastro)enteritis	6
	Cholangiohepatitis	1
	Pancreatitis	2
	Colitis	2 One with rectal prolapse One with concurrent typhlitis
	Typhlitis	1
	Bladder urolith and cystitis	2 One with calcium oxalate, positive for Feline Immunodeficiency Virus One with undetermined type of urolith
<i>Idiopathic disorders</i>	Idiopathic megacolon	1
<i>Infectious disorders</i>	Feline infectious peritonitis	6
	Toxoplasmosis	5 Including one with polycystic kidney disease
	Giardiasis	2 Including one with concurrent trichomonosis
<i>Traumatic disorders</i>	Discus hernia T9-T10	1 with concurrent HCM
	Long standing tibial fracture	1
<i>Cardiovascular disorders</i>	Hypertrophic cardiomyopathy (HCM)	1 with aortic thromboembolism and peritoneopericardial diaphragmatic hernia
<i>Neoplastic disorders</i>	Transitional cell carcinoma	1
	Hepatic benign neoplasm	1
	Hepatic and pancreatic carcinoma	1
	Hepatocellular carcinoma	1
	Intestinal adenocarcinoma and carcinomatosis	1
	Lymphosarcoma of the hard palate	1
	Mammary gland carcinoma with splenic and renal metastases	1
	Lung adenosquamous carcinoma	1
	Femoral malignant sarcoma	1 with concurrent CKD

TABLE 7 OVERVIEW OF THE DIFFERENT DISEASES DIAGNOSED IN THE GROUP OF 51 NON-ENDOCRINE SICK CATS.

The control group was composed of 94 healthy cats presented at the small animal clinic of the Veterinary Faculty of Ghent for polycystic kidney disease (PKD) screening or participating in a study on geriatric screening. All cats were submitted to a complete physical examination, a urinalysis (specific gravity, dipstick, urinary protein:creatinin ratio, urine sediment and urine culture if indicated), a complete blood count and serum biochemistry profile. Cats older than 6 years also underwent systemic blood pressure measurement, electrolytes analysis (Na, Cl, K, P), a FIV/FelV test and measurement of tT₄. Cats were considered healthy and were subsequently included if there were no significant abnormalities detected during these examinations.

For both groups, the ultrasonographic examination was performed with the cat in dorsal recumbency with only minimal manual restraint. The abdominal hairs were clipped if necessary and acoustic coupling gel was applied. Three ultrasound machines were used (ATL 3500S, Logic7* and MyLab30#) with a multifrequency linear transducer (8-14 MHz and 10-15 MHz respectively). Six ultrasonographers (supervised ECVDI senior residents) were involved in the study (five in Ghent and 1 in Alfort). The adrenal glands were imaged according to a previously described protocol².

Three 2-dimensional shapes of the adrenal gland were distinguished: bipolar (oval with a depression over the minor axis), oval (without depression) or fusiform (lower short to long axis with more pointed extremities). The echogenicity compared to the surrounding tissues (hypo-, iso- or hyperechogenic), the visualization of layers (medulla/cortex distinct or not) and the presence of hyperechoic foci (number, uni- or bilateral, with or without presence of acoustic shadowing) were also assessed by the observers during the ultrasonography. The maximum length (cranio-caudal, L) and the height of the cranial (crH) and caudal (cdH) poles (dorso-ventral) were measured on a sagittal scan of each adrenal gland, at the end of the examination by the

ultrasonographer. All images were saved and reviewed during a remote examination by the first author.

Statistical analysis was performed on adrenal gland measurements. In order to assess the effect of different covariates on the maximum length (cranio-caudal, L) and the height of the cranial (crH) and caudal (cdH) poles (dorso-ventral) of the adrenal gland, a mixed model was fitted with cat as random effect and status, breed, gender, castration and age and the two-way interactions as fixed effects. Hypothesis testing was based on the F-test at a significance level of 5%.

4.2.2 RETROSPECTIVE STUDY: CATS WITH ADRENAL DISEASE

Cats were retrospectively recruited from the database of two referral centres (Veterinary Faculty of Ghent and the CHUVA of the Veterinary school of Alfort) from 2000 to 2011. Inclusion criteria for primary hyperaldosteronism were:

- Compatible history and clinical signs (at least 2 signs from the following: weakness, cervical ventroflexion, lethargy, depression, dysphagia, systemic hypertension, polyuria, polydipsia, periuria, weight loss, diarrhoea, polyphagia, abdominal mass, dehydration)⁹.
- High plasma aldosterone concentration (> 195 pg/ml) with concurrent hypokalaemia (< 3.0 mmol/L)¹⁰.
- Abdominal ultrasound: adrenal echogenicity, shape and size available on recorded images and ultrasonography report.

Inclusion criteria for hyperadrenocorticism were:

- Compatible history and clinical signs (at least 2 signs from the following: insulin resistant diabetes mellitus, polyuria, polydipsia, fragile torn and thin skin, weight loss, lethargy, alopecia, polyphagia, potbelly, hepatomegaly, bruising, plantigrade stance)¹¹.
- Diagnosis with low dose dexamethasone suppression test (LDDS with 0.1 mg/kg IV of dexamethasone, plasma cortisol concentration >30

nmol/L after 4 and 8 hours) or urine cortisol:creatinine ratio (UCCR > 3.6×10^{-5})¹¹.

- Localization with LDDS (pituitary origin: >50% lowering of plasma cortisol concentration after 4h or 8h, adrenal origin: <50% lowering of plasma cortisol concentration after 4h or 8h) or plasma ACTH concentration (pituitary origin > 80 pg/mL, adrenal origin < 20 pg/ml) or pituitary mass on computed tomography¹¹.
- Abdominal ultrasound: adrenal echogenicity, shape and size available on recorded images and ultrasonography report.

Six cats with primary hyperaldosteronism and 4 cats with hyperadrenocorticism were included. No case of sex-hormone producing adrenal tumour could be found in the database over 10 years. The number of cats was too small to perform a relevant statistical analysis. We described the ultrasonographic appearance and size of the adrenal glands in these groups based on the review by the first author of a sagittal scan of the gland, the only plane consistently recorded and the plane used in the protocol of the prospective study. We made a descriptive comparison with the ultrasonographic appearance of the adrenal glands in the healthy and chronically sick cats described in the prospective study.

4.3 RESULTS

4.3.1 PROSPECTIVE STUDY: CHRONICALLY SICK AND HEALTHY CATS

The chronically sick feline group was composed of 9 intact males, 22 castrated males, 5 intact females and 15 neutered females. The breeds represented were Domestic Shorthair (n=29), British Shorthair (n=10), Ragdolls (n=6), Persians (n=2), Siamese (n=2) and other breeds (n=2, Scottish Fold, Maine Coon). Mean age was 7.4 years (SD = 5.5 years), median age was 5.5 years (range 0.7-19.0

years). Mean weight was 4.2 kg (SD = 1.2 kg), median weight was 4.0 kg (range 2.4-7.1 kg).

In the control group, mean age was 5.2 years (SD = 3.8 years), median age was 3.7 years (range 0.8-14.0 years). Mean weight was 4.2 kg (SD = 1.0 kg) and median weight was 4.0 kg (range 2.5-7.9 kg). There were 12 intact males, 19 castrated males, 27 intact females and 36 neutered females. It was composed of 48 Domestic Shorthairs, 21 Ragdolls, 9 British Shorthair, 5 Sphynx, 3 Maine coon, 3 Persians, 2 Norwegians and 3 other breeds (n=3, Siberian, Exotic Shorthair, Angora).

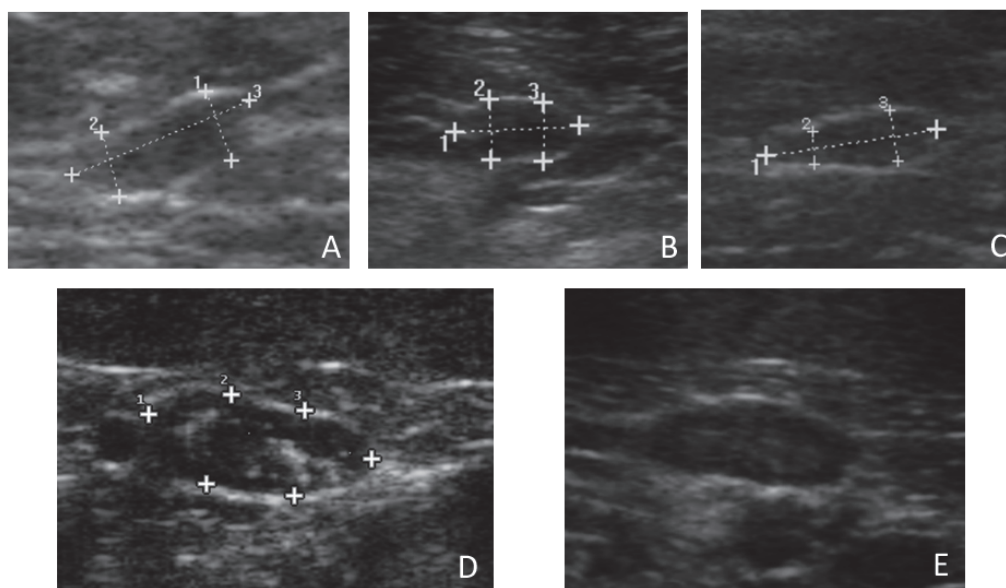


FIGURE 15 ULTRASONOGRAPHIC IMAGES OF FELINE ADRENAL GLANDS: (A) A BIPOLAR LEFT ADRENAL GLAND IN A CHRONICALLY SICK CAT (DISTANCES 1 = 3.0MM, 2 = 2.7MM, 3 = 7.7MM); (B) AN OVAL LEFT ADRENAL GLAND IN A HEALTHY CAT (DISTANCES 1 = 9.8MM, 2 = 4.7MM, 3 = 4.6MM); (C) A FUSIFORM RIGHT ADRENAL GLAND IN A CHRONICALLY SICK CAT (DISTANCES 1 = 8.1MM, 2 = 1.5MM, 3 = 2.4MM); (D) HYPERECHOIC FOCI IN A HYPOECHOIC LEFT ADRENAL GLAND IN A CHRONICALLY SICK CAT (DISTANCES 1 = 11.0MM, 2 = 4.5MM, 3 = 4.2MM); (E) CONCENTRIC LAYERS DETECTED IN A LEFT ADRENAL GLAND IN A HEALTHY CAT.

Both right and left adrenal glands could be visualized in all cats (n=145), although in 18/94 healthy cats and 12/51 chronically sick cats, the outline of one or the two adrenal glands was difficult to delineate precisely (close contact

with vasculature, small size, isoechoic to surrounding tissue). In both healthy and sick cats, the most common appearance was a well-defined, bipolar hypoechoic gland surrounded by a thin hyperechoic halo (Figure 15 A). The adrenal gland shape was less frequently recognized as fusiform or oval (Figure 15 B and C respectively).

The descriptive ultrasonographic features of the adrenal glands of the healthy and chronically sick cats are reported in Table 8. There was no difference in the shape and echogenicity of the adrenal glands between healthy and sick cats.

		Healthy cats (n=94)	Chronically sick cats (n=51)
Left adrenal 2D shape	<i>bipolar</i>	67 (71%)	31 (61%)
	<i>oval</i>	20 (21%)	13 (25%)
	<i>fusiform</i>	7 (8%)	7 (14%)
Right adrenal 2D shape	<i>bipolar</i>	58 (62%)	33 (65%)
	<i>oval</i>	17 (18%)	10 (19%)
	<i>fusiform</i>	19 (20%)	8 (16%)
Hyperechoic foci		8 cats (9%)	10 cats (20%)
	<i>bilateral</i>	4	3
	<i>LAG</i>	3	2
	<i>RAG</i>	1	5
Detectable cortico-medullary definition	<i>hyperechoic centre + hypoechoic periphery</i>	1 cat	2 cats

TABLE 8 DESCRIPTIVE ULTRASONOGRAPHIC FEATURES OF THE ADRENAL GLANDS OF THE HEALTHY AND CHRONICALLY SICK CATS.

Eighteen cats showed hyperechoic foci in the otherwise hypoechoic adrenal gland, unilaterally in 11 cats and bilaterally in 7 cats (25 glands were concerned). These hyperechoic foci were observed more often in sick cats (20%) than in healthy cats (9%). They were not creating a visible acoustic shadow distally (Figure 15 D). They demonstrated variable shapes: multiple pinpoint, linear and central, single nodular, ill-defined heterogeneous. Cranial and caudal adrenal poles were equally involved. Hyperechoic foci were

bilateral in 7/18 cats and unilateral in 11 cats (involving similarly the left and right adrenal glands).

Two distinct concentric layers could be distinguished in one healthy and two sick cats, with a hyperechoic centre and a hypoechoic periphery (Figure 15 E).

The measurements of the adrenal glands in healthy and sick cats are presented in Table 9.

Adrenal measurements from the right or the left gland were not significantly different. There was no statistical difference in adrenal gland measurements between healthy and chronically sick cats. There was no significant effect of gender or breed on adrenal gland measurements. In both groups of healthy and sick cats, there was a moderate effect of castration on the caudal height measurement of the adrenal gland ($P=0.02$). The gland was thicker in castrated cats (mean 3.7 ± 0.1 mm) than in intact cats (mean 3.4 ± 0.1 mm), in males and females combined. This mild difference is not clinically relevant. There was a small effect of age on the length in healthy cats ($P=0.03$), with a slight decrease in length with aging (estimate of the downslope = -0.1158), also not clinically relevant. There was no effect of age on the short axis measurements of the adrenal glands.

Ultrasonographic measurements in mm	Healthy cats (n=94)*	Healthy cats (n=10)#	Healthy cats (n=20)§	Chronically sick cats (n=51)*	Sick cats without endocrinopathies (n=24)@
	mean ± SD median (range)	mean ± SD	median (range)	mean ± SD median (range)	mean ± SD median (range)
Left adrenal gland	Length 10.4 ± 1.8 10.5 (5.8-14.1)	10.7 ± 0.4	8.9 (4.5-13.3)	10.8 ± 2.0 10.7 (6.9-15.5)	11.3 ± 2.8 10.6 (7.1-19.5)
	Cranial height 3.8 ± 0.8 3.8 (1.8-5.9)	4.3 ± 0.3	3.9 (3.0-5.3)	4.2 ± 0.9 4.3 (2.3-5.8)	3.8 ± 0.8 3.6 (2.8-4.7)
	Caudal height 3.6 ± 0.7 3.5 (2.3-5.5)			3.9 ± 0.8 3.9 (2.0-6.1)	
Right adrenal gland	Length 10.8 ± 1.9 10.8 (6.1-17.7)	10.7 ± 0.4	9.8 (6.7-13.7)	10.9 ± 2.0 11.0 (6.7-14.7)	9.8 ± 2.4 9.9 (5.4-13.7)
	Cranial height 3.7 ± 0.9 3.7 (1.5-6.7)	4.3 ± 0.3	3.9 (2.9-4.5)	4.0 ± 1.2 4.1 (1.5-7.5)	4.5 ± 1.0 4.2 (3.4-7.1)
	Caudal height 3.6 ± 0.7 3.7 (2.4-5.3)			3.9 ± 1.0 4.0 (1.6-6)	

TABLE 9 ULTRASONOGRAPHIC MEASUREMENTS OF THE ADRENAL GLANDS IN HEALTHY AND CHRONICALLY SICK CATS. * DATA FROM THE PRESENT STUDY, # CARTEE ET AL., 1993, § ZIMMER ET AL., 2000, @ ZATELLI ET AL., 2007.

4.3.2 RETROSPECTIVE STUDY: CATS WITH ADRENAL DISEASE

According to the inclusion criteria, six cats were diagnosed with hyperaldosteronism. They were all Domestic Shorthair cats. There were 3 castrated males, 1 intact female and 2 neutered females. The median age was 12.5 years (range 11-17 years). The median weight was 4.4 kg (range 2.6-6.8 kg). The clinicopathological findings and ultrasonographic features of the adrenal glands of cats with hyperaldosteronism are summarized in Table 10.

The most common ultrasonographic appearance was a unilateral mass (Figure 16), 4 concerning the left and 2 the right adrenal gland.

Three patterns of echogenicity were recorded: hyperechoic heterogeneous in 2 masses, hypoechoic homogeneous in 3 masses and target-like (hyperechoic centre and hypoechoic rim) in one mass. Vascular invasion was not detected in any of the cats. At the time of the ultrasonography, the contralateral gland was considered normal in size, shape and echogenicity. The histopathological analysis of the adrenal mass performed in 5/6 cats resulted in: one adrenocortical carcinoma, two adrenocortical adenocarcinomas, one adrenocortical adenoma and one adrenocortical adenomatous nodule. Malignant and benign tumours could not be differentiated according to their echogenicity pattern. The two largest masses were carcinoma and adenocarcinoma, however, one adenocarcinoma had adrenal mass measurements similar to the two benign tumours.

Histopathologically, the 3 malignant tumours were locally invasive, with cellular infiltration beyond the adrenal capsule, in the surrounding fat, even forming nodules in one cat. Phrenicoabdominal venous embolization was noticed during surgery in one of the 3 cats. None of these local invasions were diagnosed ultrasonographically. In 3/6 cats, a histopathological analysis of the contralateral adrenal gland had been performed (2 necropsy and 1 bilateral adrenalectomy): the two carcinomas were bilateral with more malignant infiltration of the gland on the side of the ultrasonographic mass but also a mild

malignant infiltration of the contralateral adrenal gland. Retrospectively these two contralateral adrenal glands were at the upper limit of adrenal measurements in healthy and chronically sick cats. The third cat had a unilateral adenoma and a normal contralateral gland, retrospectively within the mid-range of adrenal measurements of healthy and sick cats.

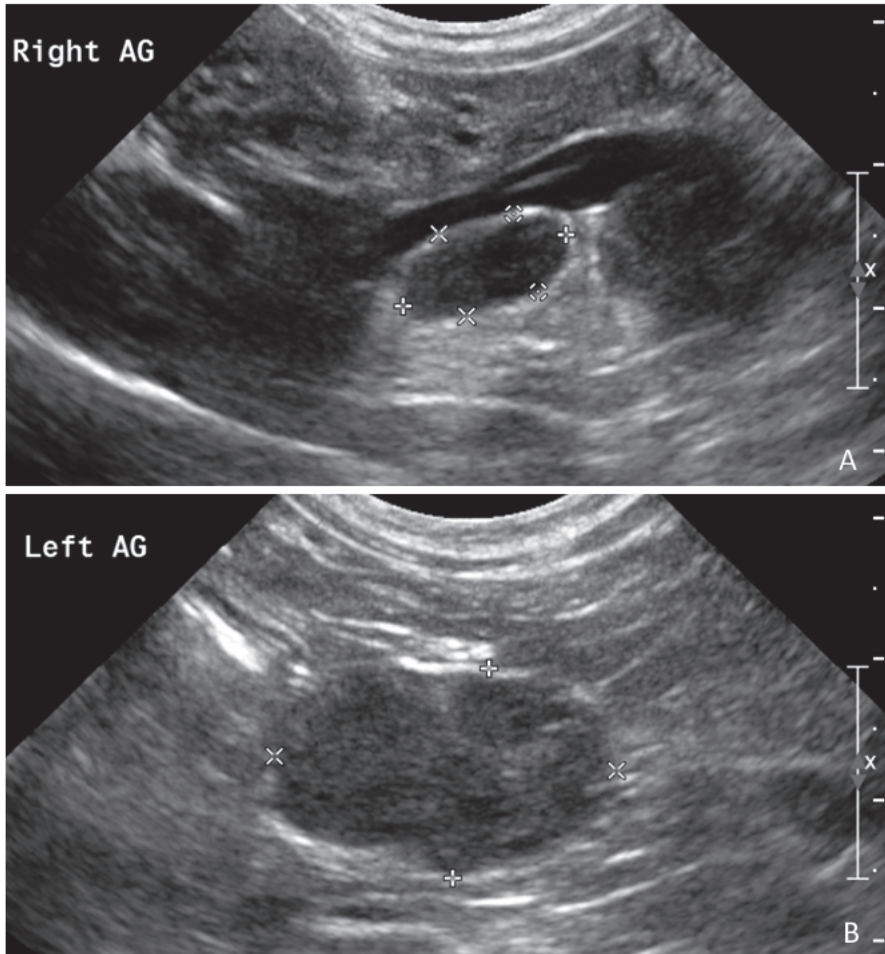


FIGURE 16 ULTRASONOGRAPHIC IMAGES OF THE RIGHT (A) AND LEFT (B) ADRENAL GLANDS IN A CAT WITH HYPERALDOSTERONISM CAUSED BY BILATERAL CARCINOMA. THE RIGHT ADRENAL GLAND HAS A NORMAL BIPOLAR SHAPE, A WELL-DEFINED CONTOUR AND A HYPOECHOIC PARENCHYMA. THE RIGHT ADRENAL GLAND LENGTH IS 12.4MM AND ITS MAXIMAL HEIGHT 6.1MM. THE LEFT ADRENAL GLAND IS ROUNDED, HYPOECHOIC, MILDLY HETEROGENEOUS WITH WELL-DEFINED BORDERS. THE LEFT ADRENAL GLAND LENGTH IS 24.3MM AND ITS HEIGHT 15.1MM.

	Clinical signs	K	PAC	PRA	Left adrenal gland ultrasound	Right adrenal gland ultrasound	Outcome	Histopathological analysis
1	PuPd, weakness, anorexia, plantigrade stance, hypothermia	2.0 mmol/L	506 pg/ml	70 fmol/L/s	24.3 x 15.1 mm Mass, hyperechoic, heterogeneous, no vascular involvement	12.4 x 6.0 mm Homogeneous, ovoid, hypoechoic	Bilateral adrenalectomy Deceased post-operatively with persistent hypokalaemia.	Bilateral adrenocortical carcinoma (LAG-RAG) Invasion of surrounding fat forming nodules
2	Acute blindness, hypothermia, retinal detachment, dyspnoea, systolic heart murmur 5/6, dehydration SBP= 280 mmHg	2.1 mmol/L	1305 pg/ml	NE	29 x 22 mm Mass, homogeneous, hypoechoic, no vascular involvement	9.7 x 5.5 mm Homogeneous, ovoid, hypoechoic	Euthanasia Necropsy	Bilateral well-differentiated adrenocortical adenocarcinoma Peripheral fat tissue infiltration Metastases in lung, liver
3	Vomiting, lethargy, anorexia, hypothermia, dehydration, bradycardia, ventricular arrhythmia	2.9 mmol/L	1592 pg/ml	NE	12.7 x 3.6 mm Homogeneous, bean-shaped, hypoechoic	15.1 x 16.5 mm Mass, hyperechoic centre and peripheral hypoechoic rim	Deceased at home after several months Necropsy	Right adrenocortical adenoma LAG: normal
4	Lethargy, PuPd, hypothermia, cervical ventroflexion, systolic heart murmur 4/6	2.2 mmol/L	204 pg/ml	<2 pg/ml	11.6 x 10.8 mm Mass, heterogeneous, hyperechoic, no vascular involvement	7.5 x 3.2 mm Elongated, hypoechoic, hyperechoic foci in caudal pole	Left adrenalectomy No recurrence after 8 months	Two left well-defined adrenocortical adenomatous nodules RAG: not examined
5	Constipation, vomiting, anorexia, PuPd, cervical flexion, dehydration, SBP = 220 mmHg	2.2 mmol/L	4755 pg/ml	NE	12 x 13 mm Mass, homogeneous, hypoechoic, no vascular involvement	8.5 x 3.5 mm Homogeneous, ovoid, hypoechoic	Left adrenalectomy and resection of phrenicoabdominal vein (emboli) Immediate improvement, lost to follow up	Left adrenocortical adenocarcinoma infiltrating the surrounding fat tissues RAG: not examined
6	Vomiting, lethargy, weight loss, abdominal mass, cervical flexion, SBP = 280 mmHg	2.4 mmol/L	395 pg/ml	NE	5.2 x 3.0 mm Homogeneous, ovoid, hypoechoic	43 x 32 mm Mass, hypoechoic homogeneous, no vascular involvement	Medical treatment. Owners declined adrenalectomy and further follow up.	Not examined.

TABLE 10 CLINICOPATHOLOGICAL FINDINGS AND ULTRASONOGRAPHIC FEATURES OF THE ADRENAL GLANDS OF CATS WITH HYPERALDOSTERONISM.

REFERENCE INTERVALS (LABORATORIES DATA AND JAVADI, 2005): POTASSIUM = 3.5-5.8 MMOL/L, PAC = 40-195 PG/ML, PRA (CAT 1) = 60-630 FMOL/L/S, PRA (CAT 4) = NO REFERENCE INTERVAL REPORTED IN CATS IN THIS LABORATORY. NE: NOT EVALUATED.

Based on our inclusion criteria, four cats were diagnosed with hyperadrenocorticism, all were of pituitary origin. No case of hyperadrenocorticism secondary to adrenocortical secreting tumour could be found in the database over 10 years. The cats with pituitary-dependent hyperadrenocorticism were all Domestic Shorthair cats. There were 1 castrated male and 3 neutered females. The median age was 14 years (range 13-15 years). The median weight was 3.2 kg (range 2.0-3.6 kg). The clinicopathological findings and ultrasonographic features of the adrenal glands of cats with pituitary hyperadrenocorticism are summarized in Table 11.

The adrenal glands had mostly a bipolar shape except one left adrenal gland that was oval (Figure 17 A). They were all heterogeneous with hyperechoic foci or hyperechoic medulla in an otherwise hypoechoic parenchyma compared to surrounding fat tissue. These foci had variable shapes and distribution: linear central, irregular central, cranial pole nodule. One cat showed an isoechoic nodule in the cranial pole of the right adrenal gland, mildly deforming the adrenal outline (Figure 17 B). In 2/4 cats, the ultrasonographic measurements of both adrenal glands were within the range of the healthy and chronically sick cats. In 2/4 cats, the short-axis measurements of both adrenal glands were in the upper range or slightly over the upper limit of the range of cranial or caudal height measurements in healthy and chronically sick cats.

A histopathological examination of the adrenal glands was performed in only one of 4 cats and another had histopathological examination of the pituitary gland.

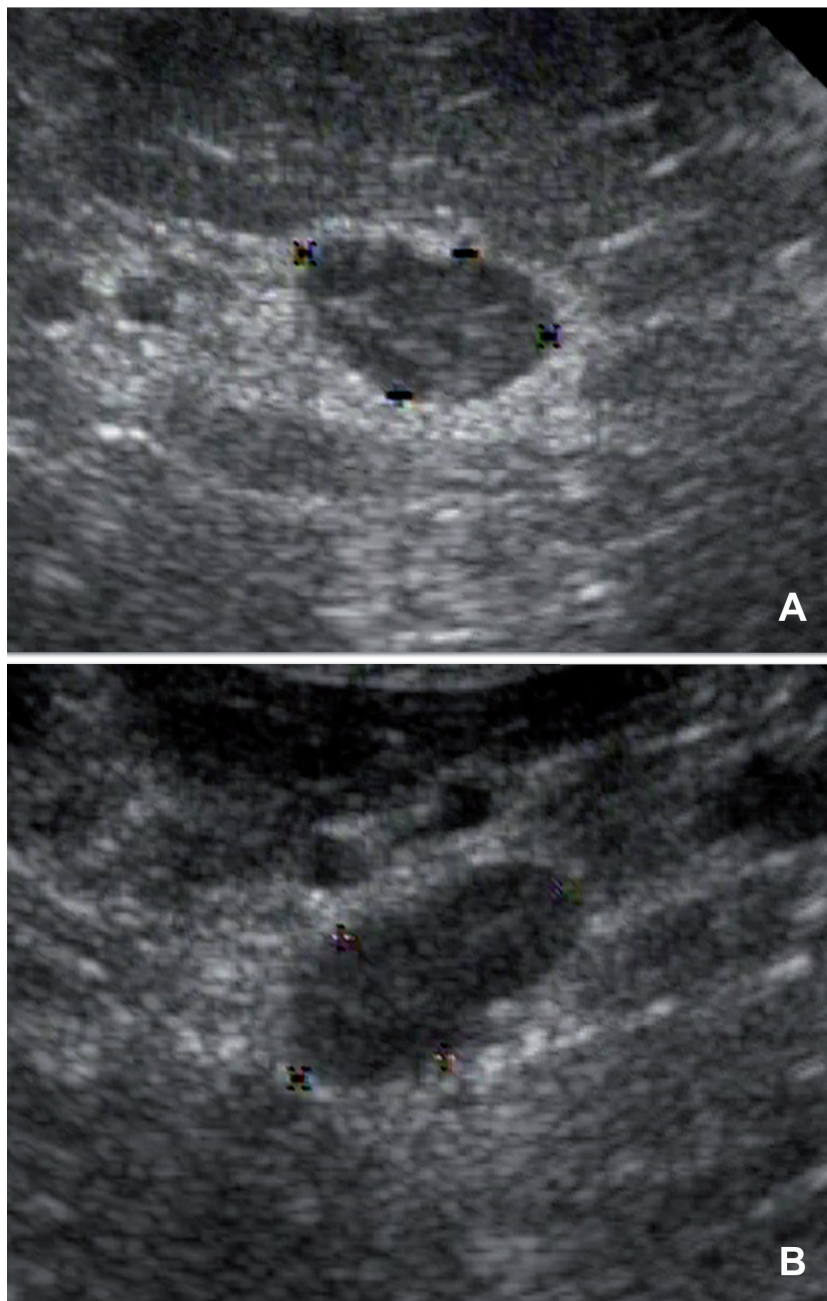


FIGURE 17 ULTRASONOGRAPHIC IMAGES OF THE LEFT (A) AND THE RIGHT (B) ADRENAL GLANDS IN A CAT WITH PITUITARY-DEPENDANT HYPERADRENOCORTICISM. BOTH ADRENAL GLANDS ARE WELL-DELINEATED, HYPOECHOIC WITH HYPERECHOIC FOCI, BIPOLAR. THEY ARE MILDLY OVER THE REFERENCE INTERVALS IN SIZE: RIGHT ADRENAL GLAND LENGTH = 13.0MM, HEIGHT = 6.4MM AND LEFT ADRENAL GLAND LENGTH = 12.2MM AND HEIGHT = 7.4MM.

	Clinical signs	HAC diagnosis	HAC origin	LAG US	RAG US	Outcome	Histo
1	Diabetes mellitus Polyuria Polydipsia Polyphagia Lethargy Subcutaneous abscess Alopecia Behavioral change	UCCR = 99,2 UCCR = 151 UCCR = 136 (<10)	Pituitary macro-adenoma (7mm) on CT IGF1 = 30.3 ng/ml GH = 0.3 mIU/L	12 x 4.5 mm Bean-shaped Hypoechoic Hyperechoic foci at the cranial pole	9.7 x 4.2 mm Bean-shaped Hypoechoic Hyperechoic foci at the cranial pole	Trilostane Caninsulin Lost to follow up	NE.
2	Diabetes mellitus Polyuria Polydipsia Fluctuating appetite Weight loss Dehydration Hypothermia Pendulous abdomen Seborrhea Systolic heart murmur 3/6	LDDS test To = 273.2 nmol/L To+4h <13.8 nmol/L To+8h = 151.8 nmol/L	LDDS test To+4h <13.8 nmol/L IGF1 = 63.2 ng/ml	14.1 x 5.2 mm Bean-shaped Hypoechoic Central hyperechoic line	12.5 x 5.7 mm Bean-shaped Hypoechoic Central hyperechoic line	Deceased during hospitalization Necropsy	Pituitary adenoma (6 mm)
3	Hyperglycemia Weight loss Anorexia Lethargy Dehydration Hypothermia Systolic heart murmur 2/6 Squamosis Telangiectasia SBP = 280 mmHg	LDDS test To = 424 nmol/L To+4h = 320 nmol/L To+8h = 180 nmol/L	LDDS test To+8h = 180 nmol/L	Short axis = 3mm Hyperechoic mineralizations	Short axis = 3 mm Hyperechoic mineralizations	Deceased 4 days after diagnosis. Necropsy was declined by the owners.	NE
4	Diabetes mellitus Lethargy Anorexia Dyspnea Dehydration Oral ulceration Seborrhea Squamosis Telangiectasia Pendulous abdomen	LDDS test To = 443.7 nmol/L To+4h = 373.2 nmol/L To+8h = 258.6 nmol/L	ACTH = 172 pg/ml	12.2 x 7.4 mm Ovoid Hypoechoic Irregular central hyperechoic foci	13.0 x 6.4 mm Bean-shaped Cranial nodule mildly deforming the outline Hyperechoic central line	Euthanasia Necropsy	Cortical hyperplastic nodules in the adrenal glands

TABLE 11 CLINICOPATHOLOGIC FINDINGS AND ULTRASONOGRAPHIC FEATURES OF THE ADRENAL GLANDS OF CATS WITH PITUITARY HYPERADRENOCORTICISM.

REFERENCE INTERVALS (LABORATORIES DATA AND FELDMAN AND NELSON, 2003): UCCR <10, LDDS: To = 13.8-149.0 NMOL/L, HYPERADRENOCORTICISM IF To+4H OR To+8H > 40 NMOL/L, PITUITARY ORIGIN IF >50% LOWERING OF PLASMA CORTISOL CONCENTRATION AFTER 4H OR 8H, IGF1 = 48.4-544.0 NG/ML, GH: NO REFERENCE VALUES IN ANIMALS IN THIS LABORATORY, ACTH: PITUITARY ORIGIN IF >80 PG/ML. NE: NOT EVALUATED.

4.4 DISCUSSION

This study confirms the ability to image and measure the feline adrenal glands with ultrasonography. Subjectively it appears easier than in dogs². In the healthy and chronically sick cats, all adrenal glands could be imaged without sedating the cats. The feline adrenal gland was surrounded by hyperechoic fat creating a very good contrast with the commonly hypoechoic gland^{1,2,12}. The presence of two distinct concentric zones in the gland was described as a hyperechoic inner zone and a hypoechoic outer zone, visible in vitro and sometimes in vivo². The histopathological significance of these two zones and their correlation with cortex and medulla were not assessed. Although a high-resolution ultrasound machine was used, two distinct zones could only be detected in one healthy and two sick cats of this study.

The three adrenal shapes described in cats, fusiform, oval or bipolar were found in the combined groups of healthy and chronically sick cats of this study, although the bipolar shape was most frequently observed in about 2/3 of the glands². The oval and fusiform shapes were equally observed in the last third of glands. The shape of the adrenal gland in a sagittal plane appeared influenced by the exact ultrasonographic scan plane. As in dogs, the feline adrenal glands are often slightly oblique compared to the sagittal plane of the body with a more lateral cranial pole and more medial caudal pole¹³. The adrenal gland appeared more bipolar if the scanning angle allowed a real sagittal plane of the gland to be performed or more oval if the plane was more sagittal to the body.

The presence of hyperechoic areas in the adrenal gland creating an acoustic shadowing was already described in healthy adult cats and interpreted as microscopic calcifications in up to 30% of the normal adult cats^{12,14}. In the present study, these areas were not associated with acoustic shadowing. The different hypotheses for this appearance are too small mineralization to create a visible acoustic shadow, fat deposition or small haemorrhage. The cats with these hyperechoic foci were of all age, gender and breed and they had a

moderate weight. Proportionally, more sick cats (20%) had hyperechoic foci than healthy cats (9%). More frequent hyperechoic foci were also described in hyperthyroid cats compared to healthy old cats⁶. The latter study proposed the hypothesis of a relationship between the hyperechoic foci and a stimulation of the hypothalamic-pituitary-adrenal axis by a chronic stress in the case of the chronically sick cats. Moreover, all 4 cats with hyperadrenocorticism presented hyperechoic foci, hyperechoic central line or mineralization in both adrenal glands.

As the cats examined at a veterinary hospital are usually sick, the influence of chronic illness on the adrenal ultrasonography is an important factor to check for. Health status and hospitalization were indeed reported to influence the urine cortisol:creatinin ratio in cats but not the dexamethasone suppression test and the adrenocorticotrophic hormone stimulation test^{5,15-17}. Comparing with previous studies in healthy cats, the adrenal measurements were more recently described in a small group of 24 sick cats without endocrine diseases¹⁻³. They appeared subjectively similar in size. The limitations of the previous studies were: a small number of cats, the absence of a control group in the study on sick cats and the lack of standardization of the ultrasonographic protocol. Therefore, the possible absence of influence of general non-hormonal non-adrenal illness on adrenal gland ultrasound dimensions was explored in the present study, in a large group of cats, applying a standardized ultrasound protocol using high-resolution ultrasound equipment of the same generation. All adrenal measurements had the tendency to be larger in chronically ill cats compared to healthy cats, however none of them were statistically significant. From a clinical point of view, the ultrasonographic measurements of the adrenal glands can be considered similar in healthy and chronically sick cats. Therefore, the reference interval of adrenal measurements can be inferred from the values obtained in a combined group of healthy and chronically sick cats (mean \pm 1.96*SD): the adrenal length is 6.9 to 14.4 mm; the cranial height

is 2.0 to 5.7 mm; the caudal height is 2.2 to 5.2 mm. The mean, median and range of adrenal measurements obtained in this study were comparable with the previous studies¹⁻³. In the present study, a higher upper limit was however noticed in length and height measurements (Table 9). Differences in length may depend on the visibility of the gland, particularly the right adrenal gland. As described previously for the shape of the gland, it appeared necessary to scan in a sagittal plane of the adrenal gland instead of in a sagittal plane of the body, to obtain the longest cranio-caudal length. An *in vitro* study with ultrasonographic examination in different imaging planes of isolated adrenal gland specimens should however be performed to test this hypothesis. The height measurement of this study and the thickness measurement previously described could also represent two different anatomical distances that could explain the difference in mean thickness. The authors strictly measured the dorso-ventral height of each gland to assess a more precise and repeatable measurement of the gland. However, considering the measurement accuracy of the ultrasonography machines, these values are most likely rather comparable. According to previous studies, there was no effect of bodyweight, body surface area, body condition score, gender and side on the ultrasonographic measurements of adrenal glands in healthy or chronically sick cats^{2,3}. As previously described, we did not find any difference between male and female cats². However statistically, the adrenal glands of castrated cats were larger than those of intact cats in both groups of healthy and sick cats. A study described an increased aldosterone to renin activity ratio in healthy neutered cats compared to intact cats¹⁸. The authors speculated that oestrogens and luteinizing hormone (LH) could increase the expression of the adrenal receptors for angiotensin II and then increase the aldosterone production. This hypothesis is comparable to the pathogenesis of the hyperadrenocorticism in ferrets¹⁹. In ferrets, adrenal hyperplasia is suspected to be due to an absent negative feedback from the gonadal steroids on the pituitary gland and then an

increase in circulating gonadotropin levels. Gonadotropins stimulate the expression of LH receptors in the adrenal cortex, leading to overproduction of sex-steroid hormones and adrenal hyperplasia. By reduced negative feedback from absent gonadal steroid due to castration, an enlargement of the adrenal glands may be explained in castrated cats. It has however a low clinical significance given the difference in adrenal size is mild compared to resolution of ultrasound equipment. The effect of aging on adrenal gland size had been studied in humans, dogs and rats²⁰⁻²³. These studies commonly described an adrenal hyperplasia and an increase in adrenal gland size or volume with aging. This age effect was detectable by ultrasound in dogs^{24,25}. In one study, a weak but significant relationship between age and adrenal length was reported ($P=0.009$). In the other study, a significant relationship was obtained between age and left adrenal length ($P=0.0388$) and width ($P=0.0019$). According to these data in dogs and clinical experience of rounder and larger adrenal glands in older cats, the weak statistical relationship between age and adrenal length was surprising as the adrenal length decreased with aging. However if they are not thicker, the decrease in length could explain the rounder shape of feline adrenal gland with aging. This apparent rounding of the gland should not have any clinical consequence concerning adrenal disease diagnosis if the short axis of the gland is measured accurately with ultrasound.

One limitation of the prospective study in healthy and chronically sick cats is the absence of histopathological analysis of the adrenal glands. For ethical reasons, the characterization of the health status and the exclusion of endocrine diseases in healthy and chronically sick cats were limited to clinicopathologic data.

In the retrospective analysis of data covering 11 years, the ultrasonographers and the equipment were variable, although the most recent cases were examined by some of the ultrasonographers of the prospective study. This represents a limitation in the remote interpretation the ultrasonographic

images of adrenal glands as the scan plane could be inaccurate, thus changing the shape or the size of the gland as previously hypothesized. In reviewing the images of adrenal glands included in the retrospective study, image quality was checked to allow comparison with the prospective study data. However inaccuracy in scan plane is difficult to judge on a fixed image and remains possible.

In the six cases of hyperaldosteronism of this study, the ultrasonographic findings were mainly a unilateral mass of various echogenicity and echotexture. The size of the mass was obviously larger than the size of adrenal glands in healthy or chronically sick cats. Vascular invasion by the mass was never detected with ultrasound. The five cases with histopathological diagnosis were compared with the 22 cases of hyperaldosteronism reported in the literature with ultrasound description and histopathological or cytological diagnosis^{10, 26-33}. As in our study, there are an equal proportion of benign and malignant adrenal neoplasms in hyperaldosteronism. The size (10 to 47 mm) or the other ultrasonographic descriptive features of the mass (echogenicity, homogeneity and shape) did not allow the differentiation between benign and malignant adrenal lesions. As severe compression or invasion of caudal vena cava could be difficult to differentiate, even the appearance of adjacent vessels was not more specific.

Two of the six cases of hyperaldosteronism of this study were initially diagnosed as unilateral adrenal mass with ultrasonography and further confirmed as bilateral carcinoma after necropsy. Another similar case report of unilateral mass on ultrasound further diagnosed as bilateral adenoma on necropsy has been reported²⁹. As unilateral adrenalectomy or unilateral fine – needle aspirate of the apparently abnormal gland are most often described in the literature, the contralateral gland is most often not sampled and then rarely confirmed as normal with histopathological analysis. The real prevalence of bilateral involvement in adrenocortical tumour is most likely underestimated.

Additionally, two cases of confirmed bilateral hyperplasia did not show any ultrasonographic abnormalities¹⁰. Consequently, a normal contralateral gland to mass or bilateral normal adrenal glands on ultrasound does not exclude a benign or malignant adrenocortical infiltration. This finding can be relevant if surgical treatment is considered, as unilateral adrenalectomy may not be successful if the other gland is also infiltrated or hyperplastic.

With the inclusion criteria of this study, only four cats with pituitary-dependent hyperadrenocorticism (PDH) could be recruited. Moreover, no adrenal-dependent hyperadrenocorticism (ADH) could be found in the database at all. This is most likely reflecting the higher frequency of PDH (40/50) compared to ADH (10/50) reported in the literature^{11, 34-42}. One limitation is the low number of histopathological analysis of adrenal glands and pituitary glands in this group, but the diagnosis and origin of hyperadrenocorticism was based on biological testing. We compared the four cases of the present study with the 50 cases reported with ultrasonographic description and definite diagnosis and location of hyperadrenocorticism^{11, 34-42}. In PDH, the majority of the cases showed bilateral adrenomegaly, although a few cases had a normal adrenal size compared to the reference ranges. In ADH, the main finding is a unilateral mass with a normal contralateral gland or no visualization of the contralateral gland. As ultrasonographic protocol and normal data about imaging the feline adrenal glands are getting more precise, the ultrasonographic detection and description of adrenal glands may become more consistent. For instance, adrenal size and asymmetry may help in the suspicion of endocrine diseases in cats, although the diagnosis is invariably based on results of endocrinologic testing. Indeed atypical cases like bilateral adrenal tumours or asymmetrical adrenal hyperplasia would stay misleading with imaging.

4.5 CONCLUSION

The ultrasonographic examination of feline adrenal glands is more easily performed with modern high-resolution ultrasound device. The typical hypoechoic appearance of the gland surrounded by hyperechoic fat makes it recognizable. A sagittal plane of the gland, not in line with the aorta, is necessary to obtain the highest adrenal measurements and the most accurate shape of the gland. There is no significant difference in adrenal measurements between healthy and chronically sick cats, although the adrenal glands of sick cats presented more often hyperechoic foci. There was no clinically significant effect of age, gender, castration, breed or side on the adrenal gland measurements. Cats with hyperaldosteronism most frequently presented with unilateral severely enlarged adrenal gland. However a normal contralateral gland does not preclude a contralateral infiltration in benign or malignant adrenal neoplasms. The ultrasonographic appearance of the adrenal glands could not distinguish benign from malignant lesions. The ultrasonographic appearance of pituitary-dependent hyperadrenocorticism is mainly a symmetrical adrenal enlargement, however a substantial number of cases were within the normal range of adrenal size established in healthy and chronically sick cats (caudal height < 5,2 mm).

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5 ULTRASONOGRAPHIC MEASUREMENTS OF ADRENAL GLANDS IN CATS WITH HYPERTHYROIDISM

Feline hyperthyroidism is potentially associated with exaggerated responsiveness of the adrenal gland cortex. The adrenal glands of 23 hyperthyroid cats were examined ultrasonographically and compared to the adrenal glands of 30 control cats. Ten hyperthyroid cats had received antithyroid drugs until 2 weeks before sonography, the other 13 were untreated. There was no difference in adrenal gland shape between healthy and hyperthyroid cats: bean-shaped, well-defined, hypoechoic structures surrounded by a hyperechoic halo in 43/60 (71.6%) healthy cats and 34/46 (73.9%) hyperthyroid cats; more ovoid in 13/60 (21.6%) healthy cats and 9/46 (19.6%) hyperthyroid cats while more elongated in 4/60 (6.7%) healthy cats, 3/46 (6.5%) hyperthyroid cats. Hyperechoic foci were present in 9/23 (39.1%) hyperthyroid cats and 2/30 (6.7%) healthy cats. The adrenal glands were significantly larger in hyperthyroid cats, although there was overlap in size range. The mean difference between hyperthyroid cats and healthy cats was 1.6 and 1.7 mm in left and right adrenal gland length, 0.8 and 0.9 mm in left and right cranial adrenal gland height and 0.4 and 0.9 mm in left and right caudal adrenal gland height. There was no significant difference between the adrenal gland measurements in treated and untreated hyperthyroid cats. The adrenomegaly was most likely associated with the hypersecretion of the adrenal cortex documented in hyperthyroid cats. Hyperthyroidism should be an alternative differential diagnosis to hyperadrenocorticism, hyperaldosteronism and acromegaly in cats with bilateral moderate adrenomegaly.

Adapted from: Combes A, Vandermeulen E, Duchateau L, Peremans K, Daminet S, Saunders J. **Ultrasonographic measurements of adrenal glands in cats with hyperthyroidism.** *Vet Radiol Ultrasound* 2012; 53(2): 210-6.

5.1 INTRODUCTION

Illness and stress influence the hypothalamic-pituitary-adrenal axis¹⁻³. However, little data are available about the ultrasonographic appearance of adrenal glands in sick cats. In 24 sick cats without endocrine disease there was no difference in adrenal gland size compared to normal cats⁴⁻⁶. Also, adrenal glands in diabetic cats were not enlarged compared with those of healthy cats⁷. Hyperthyroidism in cats may be associated with exaggerated responsiveness of the adrenal cortex as adrenocortical hyperplasia was found in one third of hyperthyroid cats⁸. Hyperthyroid cats had higher basal, post-dexamethasone and post-ACTH cortisol concentrations than other sick cats ($P=0,06$)¹. An abnormally high urinary cortisol to creatinine ratio was also found in hyperthyroid cats⁹. We have personally observed that the adrenal glands of hyperthyroid cats were uniformly larger and rounder than in other sick cats. This appearance could be misinterpreted as bilateral adrenal hyperplasia or bilateral adrenal tumour. Indeed, clinical signs observed with hyperthyroidism and adrenal disease can overlap. It can also be misleading when adrenal disease is suspected clinically and then evaluated ultrasonographically in cats with confirmed hyperthyroidism.

Our aim was to evaluate the ultrasonographic features of the adrenal glands in hyperthyroid cats.

5.2 MATERIALS AND METHODS

Twenty-three hyperthyroid cats were hospitalized for radioactive iodine treatment from August 2010 to December 2010. The diagnosis of hyperthyroidism was based on clinical signs, such as weight loss, polyphagia, hyperactivity or tachycardia, a high serum thyroxin concentration (tT_4) and a pertechnetate scan with increased thyroid:salivary gland ratio ($>1:1$). The hyperthyroid cats were divided in two groups: 10 cats that received antithyroid drugs and 13 cats that never received antithyroid medication.

Antithyroid treatment duration was 1 to 5 months (mean 2 months). The treatment was discontinued systematically 2 weeks before the examination.

The 10 treated cats ranged in age from 8.7 to 16.0 years, with a mean of 11.7 (SD \pm 2.1) years and a median of 11.5 years. Weight ranged from 2.2 to 7.8 kg with a mean of 4.0 kg (SD \pm 1.7) and a median of 3.6 kg. There were 2 neutered males, 5 neutered females and 3 intact females. All treated cats were Domestic Shorthairs.

The 13 untreated cats ranged in age from 9.3 to 15.2 years old with a mean of 11.7 (SD \pm 1.9) years and a median of 12.0 years. Weight ranged from 2.4 to 6.0 kg with a mean of 4.0 kg (SD \pm 1.0) and a median of 4.0 kg. There were 6 neutered males, 1 intact male, and 6 neutered females. There were 12 Domestic Shorthairs and one Siamese.

The owner and the referring veterinarian were contacted by phone and email 4 to 6 months after the initial examination to assess the clinical evolution of hyperthyroidism and to exclude the clinical appearance of adrenal disease. The tT_4 was also controlled and transmitted by the referring veterinarian to assess the biological resolution of hyperthyroidism after radioactive iodine treatment. Thirty healthy privately-owned cats comprised the control group. The inclusion criteria were absence of clinical abnormalities, normal complete blood count biochemistry and urinalysis results, negative FIV and FeLV tests, normal tT_4 and normal systemic blood pressure. The age of the cats in the control group ranged from 6.0 to 14.0 years with a mean of 10.0 (SD \pm 2.3) years and a median age of 10 years. Weight ranged from 2.9 to 7.9 kg, with a mean of 4.3 kg (SD \pm 1.2) and a median of 4.0 kg. There were 6 neutered males and 24 neutered females. Breeds were 28 Domestic Shorthairs, one Ragdoll and one Maine Coon.

To minimize stress in the hyperthyroid cats, the ultrasonographic examination was performed during preparation for the radioactive iodine treatment, taking advantage of the recovery of the anaesthesia for the pertechnetate scan

(propofol, Propovet®, Abbott Laboratories, Queenborough, Kent, UK). Conventional radiation safety measures were used. The ultrasonographer wore latex gloves and a dosimeter badge. The ultrasound machine and the transducer were protected by a plastic sheath and latex glove, respectively. As there was no indication for anaesthesia in the healthy cats, the ultrasonographic exam was performed with minimal manual restraint.

All measurements were performed with the cats in dorsal recumbency. The abdominal hair was shaved and acoustic coupling gel was applied. The ultrasonography was done by one of the two last-year residents with a MyLab30 ultrasound machine (Esaote, Firenze, Italy) and a multifrequency linear transducer (10-15 MHz). The diagnosis of each cat was known by the ultrasonographer as the hyperthyroid cats were examined in the nuclear medicine department whereas the control cats were examined in the small animal clinic. The adrenal glands were imaged using a previously described technique⁶. The maximum length (cranio-caudal, L) and maximum height (dorso-ventral) at the cranial (crH) and caudal (cdH) poles were measured on a longitudinal sagittal scan for each adrenal gland. The measurements were made on a still image during the real-time examination. The shape of the adrenal gland (bean-shaped, ovoid or elongated), its echogenicity compared to the surrounding fat (hypo-, iso- or hyperechoic), the visualization of layers (medulla/cortex distinct or not) and the presence of hyperechoic foci (number, uni- or bilateral, with or without presence of acoustic shadowing) were also recorded. The interpretation of ultrasonographic images was made during the real-time scanning and reviewed on the still images later to obtain a consensus. The analysis of the three response variables (length, cranial height and caudal height) is based on a linear mixed model with animal as random effect. First, the effect of side, gender, age and weight on the response variables was tested within each disease group (healthy, treated hyperthyroid or untreated hyperthyroid) using the F-test and the 5% significance level. Second, the three

disease status groups were compared first globally using the F-test at the 5% significance level; next groups were compared pairwise using Bonferroni's technique to adjust for multiple comparisons.

5.3 RESULTS

Both adrenal glands could be visualized in all 30 control cats (Figure 18). The most common ultrasonographic appearance was a well-defined, bean-shaped hypoechoic structure surrounded by a thin hyperechoic halo: 22/30 (73.3%) left and 21/30 (70.0%) right adrenal glands. It appeared as a more ovoid structure in 7/30 (23.3%) left and 6/30 (20.0%) right adrenal glands and had an elongated shape in 1/30 (3.3%) left and 3/30 (10.0%) right adrenal glands. A hyperechoic focus, not associated with acoustic shadowing, was detected in 2 cats (6.6%, one bilateral and one unilateral). The adrenal cortex-medulla could not be differentiated in any control cat.

In the control group (Table 12), mean length of the left adrenal gland was 10.1 mm (SD 2.2 mm) and mean length of the right adrenal gland was 9.9 mm (SD 1.6 mm). Mean crH of the left adrenal gland was 4.1 mm (SD 0.9 mm) and mean crH of the right adrenal gland was 3.8 mm (SD 0.9 mm). Mean cdH of the left adrenal gland was 3.8 mm (SD 0.9 mm) and mean cdH of the right adrenal gland was 3.7 mm (SD 0.7 mm).

There was no significant difference between the ultrasonographic measurements of the left and the right adrenal glands. There was no statistical effect of gender, breed, age or weight on the adrenal gland measurements.

Untreated hyperthyroid cats (n=13)			Hyperthyroid cats with antithyroid drug (n=10)		Control cats (n=30)		Difference of combined measurements of left and right adrenal glands between groups (mean (SD) in mm)
	LAG	RAG	LAG	RAG	LAG	RAG	
Length (mm)	11.1 (9.2-14.5)	11.7 (7.9-19.4)	11.1 (9.7-12.4)	11.8 (10.4-14.5)	10.1 (5.8-14)	10.1 (6.1-13)	Untreated hyperthyroid vs. treated (0,62) Untreated hyperthyroid vs. control (0,49)* Treated hyperthyroid vs. control (0,54)*
Cranial height (mm)	4.4 (3.5-6.5)	4.7 (3.6-6.2)	4.8 (3.8-6.2)	4.9 (2.8-5.9)	4.0 (2.5-5.9)	3.8 (1.5-5.5)	0,07 (0,31) 0,80 (0,25)* 0,87 (0,27)*
Caudal height (mm)	4 (3-5.2)	4.2 (3.1-5.4)	4.5 (3.8-6.3)	4.4 (3.3-5.8)	3.7 (2.3-5.2)	3.5 (2.4-5.2)	0,50 (0,28) 0,41 (0,22) 0,92 (0,24)*
The bolded measurements represent the AG measurements of hyperthyroid cats which are statistically different from the AG measurements of control cats (P<0,05). * Adrenal measurements which are significantly different between the two compared groups (P<0,05).							

TABLE 12 COMPARISON OF THE ULTRASONOGRAPHIC MEASUREMENTS OF THE ADRENAL GLANDS IN HYPERTHYROID AND CONTROL CATS.

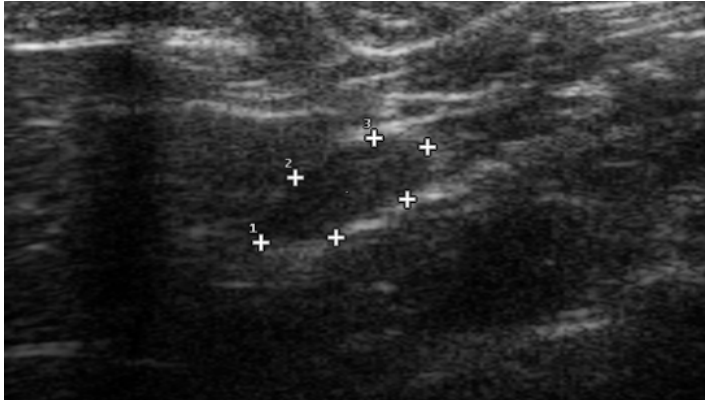


FIGURE 18 SAGITTAL ULTRASONOGRAPHIC IMAGE OF THE RIGHT ADRENAL GLAND OF A HEALTHY CAT. NOTE THE HYPOECHOIC WELL-DEFINED OVOID GLAND SURROUNDED BY A HYPERECHOIC HALO. CORRESPONDING MEASUREMENTS ARE D1=9.6 MM (LENGTH) ; D2=3.6 MM (CRANIAL HEIGHT) ; D3=3.5 MM (CAUDAL HEIGHT). 24x19MM.

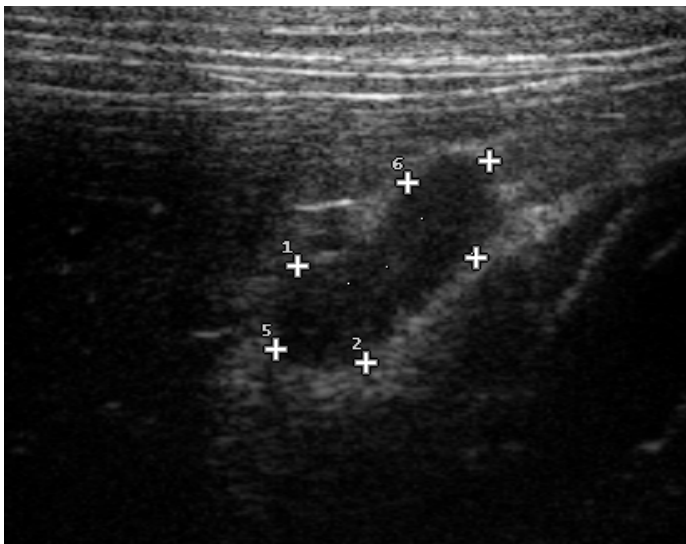


FIGURE 19 SAGITTAL ULTRASONOGRAPHIC IMAGE OF THE RIGHT ADRENAL GLAND OF A HYPERTHYROID CAT. NOTE THE BEANSHAPED HYPOECHOIC WELL-DEFINED GLAND SURROUNDED BY A HYPERECHOIC HALO. CORRESPONDING MEASUREMENTS ARE D5=5.7 MM (LENGTH); D1-2=5.2 MM (CRANIAL HEIGHT); D6=14.5 MM (CAUDAL HEIGHT). 34x27MM.

Both adrenal glands could be visualized in all 23 hyperthyroid cats (Figure 18). In both treated and untreated hyperthyroid cats, the most common ultrasonographic appearance was a well-defined, bean-shaped hypoechoic

structure surrounded by a thin hyperechoic halo: 19/23 (82.6%) left and 15/23 (65.2%) right adrenal glands. The adrenal gland appeared as a more ovoid structure in 4/23 (17.4%) left and 5/23 (21.7%) right adrenal glands and, it was elongated in 3/23 (13.0%) right adrenal glands and no left adrenal glands. A hyperechoic focus, not associated with acoustic shadowing, was observed in 8/13 (61.5%) untreated hyperthyroid cats (3 bilateral and 5 unilateral) and in 1/10 (10.0%) treated hyperthyroid cat (bilateral). In 3 hyperthyroid cats (1/10 treated and 2/13 untreated), two concentric layers were differentiated with a hyperechoic central zone and a hypoechoic peripheral zone.

In treated hyperthyroid cats (Table 12), mean length of the left adrenal gland was 11.1 mm (SD 1.0 mm) and mean length of the right adrenal gland was 12.2 mm (SD 1.7 mm). Mean crH of the left adrenal gland was 4.9 mm (SD 0.9 mm) and mean crH of the right adrenal gland was 4.7 mm (SD 0.9 mm). Mean cdH of the left adrenal gland was 4.7 mm (SD 0.9 mm) and mean cdH of the right adrenal gland was 4.5 mm (SD 0.8 mm).

In untreated hyperthyroid cats (Table 12), mean length of the left adrenal gland was 11.5 mm (SD 1.7 mm) and mean length of the right adrenal gland was 12.0 mm (SD 3.0 mm). Mean crH of the left adrenal gland was 4.5 mm (SD 0.9 mm) and mean crH of the right adrenal gland was 4.9 mm (SD 0.7 mm). Mean cdH of the left adrenal gland was 4.1 mm (SD 0.7 mm) and mean cdH of the right adrenal gland was 4.1 mm (SD 0.8 mm).

There was no significant difference between the ultrasonographic measurements of the left and the right adrenal glands. There was no statistical effect of gender or breed on the adrenal gland measurements. In the treated hyperthyroid cats, there was no statistical significant effect of weight and age on the adrenal gland measurements. In the untreated hyperthyroid cats, there was a significant positive association between weight and adrenal gland length ($P=0.006$). There was no significant statistical effect of weight on the cranial

and caudal height. There was a weak but significant positive linear correlation between age and adrenal gland cranial height ($P=0.04$). There was no significant statistical effect of age on the length and the caudal adrenal gland height. There was no significant difference between the adrenal gland measurements in the treated and untreated hyperthyroid cats.

Four to six months after the initial presentation, 18/23 cats had complete clinical resolution of hyperthyroidism (1 cat with an upper-limit tT_4 , 10 cats with normal tT_4 , 3 cats with low tT_4 and 4 cats without tT_4 tested). One of these cats died unexpectedly after resolution of hyperthyroidism. Two cats remained clinically and biologically hyperthyroid after treatment, one of the two died unexpectedly. Two cats died for unrelated reasons early in the follow-up period, before assessment of hyperthyroidism status. One cat was lost for follow-up.

The treated and untreated hyperthyroid cats had age and weight comparable to the control cats. There was no difference in shape and echogenicity of the adrenal glands between the 3 groups. As there was no statistically significant difference between left and right adrenal gland measurements in each group, the comparison between the 3 groups was done combining the measurements of left and right adrenal glands. The three measurements of the adrenal gland were significantly larger in treated hyperthyroid cats and in untreated hyperthyroid cats compared to control cats (Table 12). Comparing control and treated hyperthyroid cats, the difference in length was 1.6 mm ($SD=0.5$; $P=0.0038$), the difference in cranial height 0.9 mm ($SD=0.3$; $P=0.0021$) and the difference in caudal height 0.9 mm ($SD=0.2$; $P=0.0004$). Comparing control and untreated cats, the difference in length was 1.7 mm ($SD=0.5$; $P=0.0010$), the difference in cranial height 0.8 mm ($SD=0.2$; $P=0.0021$). The difference in caudal height was not statistically significant ($P=0.0665$).

5.4 DISCUSSION

In our feline population, the adrenal glands had a similar ultrasonographic shape in healthy cats vs. hyperthyroid cats. However, the adrenal glands in hyperthyroid cats had larger craniocaudal and dorsoventral measurements. They also had more hyperechoic foci in their parenchyma, particularly in untreated hyperthyroid cats.

The increased frequency of hyperechoic foci in the adrenal glands of the hyperthyroid cats was unexpected. The presence of hyperechoic areas in the adrenal glands that create acoustic shadowing has been described in healthy adult cats^{10,11}. Potential causes are fat deposition, small haemorrhage or microscopic calcifications. In this study, these areas were not associated with acoustic shadowing, probably because of their small size. In humans, heterogeneous adrenal masses are reported in massive adrenal haemorrhage, which can be hyper- or hypoechoic depending on the age of the haemorrhage, following severe critical illness or exogenous ACTH or steroids administration¹². To our knowledge, there is no histopathological characterization of these hyperechoic foci in the cat.

According to the frequency of hyperechoic foci and the enlargement of the adrenal glands in hyperthyroid cats, it could be hypothesized that they are linked with the stimulation of the hypothalamic-pituitary-adrenocortical axis. In hyperthyroid humans, the functional adrenal capacity of the adrenal cortex is nearly double for cortisol production per unit of time. There are not only more cortisol secretory episodes but also the amount of cortisol is increased per secretory episode¹³. This increase in cortisol production is caused by increased pituitary secretion of ACTH¹⁴. A significant increase in ACTH release induced by thyroid hormone excess led to symmetric enlargement of the adrenal glands in rats¹⁵. Conversely, thyrotoxicosis in hypophysectomised rats did not cause adrenocortical enlargement. Although the adrenal gland size may represent the adrenocortical secretory activity, it does not correlate with the

elevation of the circulating corticosteroid hormones. Human patients with thyrotoxicosis have a significantly increased plasma clearance of cortisol, which is associated with an increased adrenocortical cortisol production¹⁶. In our cats, it is possible that circulating thyroid hormone stimulated cortisol clearance and ACTH release, that caused a secondary bilateral enlargement of the adrenal cortex¹⁷. The higher urinary cortisol to creatinine ratio has been documented in hyperthyroid cats⁹. However, the trend for the basal, post-dexamethasone and post-ACTH cortisol concentrations to be higher was weak in a group of 9 hyperthyroid cats¹. There is no assessment of ACTH concentration in hyperthyroid cats, which would be more informative about the stimulation of the hypothalamic-pituitary-adrenocortical axis by the thyroid hormones. Additionally, adrenocortical hyperplasia was also reported as a post-mortem finding in one third of hyperthyroid cats⁸. Hyperthyroid cats also exhibit an exaggerated response to minor stresses. This may be associated with an activation of the hypothalamic-pituitary-adrenocortical axis and adrenal gland enlargement⁹.

The adrenal measurements were not significantly different in hyperthyroid cats that received antithyroid treatment compared to untreated cats. As methimazole decreases the tT₄ level, it is possible that activation of the hypothalamic-pituitary-adrenocortical axis would also be decreased by this treatment. Consequently, the hyperplastic effect on adrenal glands would also decrease. . In hyperthyroid cats, the mean residence time of methimazole after oral administration ranged from 92 to 345 minutes and the serum terminal half-life from 1.1 to 3.7 hours.^{18,19} There was also a decrease in the elimination half-life after multiple dose administration of methimazole for two weeks to normal cats. Considering the 2-week delay between the withdraw of the oral methimazole and the ultrasonographic examination, the cats that previously received methimazole could have been considered as untreated hyperthyroid cats. As there is no kinetic study about the time necessary for the adrenal gland

to enlarge or decrease in size relative the activation or inhibition, respectively, of the hypothalamic-pituitary-adrenocortical axis, the treated and untreated hyperthyroid cats were evaluated separately even if a similar adrenal gland size was expected.

			Groups of cats			
			Sick cats without endocrinopathy (n=24) #	Healthy cats (n=10)*	Healthy cats (n=20)†	Healthy old cats (n=30)§
LAG	Length (mm)	Mean (SD)	11.3 (2.8)	10.7 (0.4)	-	10.1 (2.2)
		Median (range)	10.6 (7.1-19.5)	-	8.9 (4.5-13.3)	10.1 (5.8-14)
	Thickness (mm)	Mean (SD)	3.8 (0.8)	4.3 (0.3)	-	Cranial : 4.1 (0.9) Caudal : 3.8 (0.9)
		Median (range)	3.6 (2.8-4.7)	-	3.9 (3.0-5.3)	Cranial : 4.0 (2.5-5.9) Caudal : 3.7 (2.3-5.2)
RAG	Length (mm)	Mean (SD)	9.8 (2.4)	10.7 (0.4)	-	9.9 (1.6)
		Median (range)	9.9 (5.4-13.7)	-	9.8 (6.7-13.7)	10.1 (6.1-13)
	Thickness (mm)	Mean (SD)	4.5 (1.0)	4.3 (0.3)	-	Cranial : 3.8 (0.9) Caudal : 3.7 (0.7)
		Median (range)	4.2 (3.4-7.1)	-	3.9 (2.9-4.5)	Cranial : 3.8 (1.5-5.5) Caudal : 3.5 (2.4-5.2)

TABLE 13 ULTRASONOGRAPHIC MEASUREMENTS OF THE ADRENAL GLANDS IN HEALTHY CATS REPORTED IN THE LITERATURE AND FROM THE PRESENT STUDY.

ZATELLI AND OTHERS 2007, * CARTEE AND FINN-BODNER 1993, † ZIMMER AND OTHERS 2000, § DATA FROM THE PRESENT STUDY

The adrenal measurements of our control group are similar to those reported previously (Table 13)^{4-6,11}. Compared to the mean ultrasonographic measurements of normal feline adrenal glands, the adrenal enlargement detected in hyperthyroid cats represents an increase in 16 and 17% of the length of the left and right adrenal glands, respectively, and an increase in 21-22% and 11-24% of the height of the left and right adrenal glands, respectively. The clinical consequence of a 20% adrenal gland enlargement due to hyperthyroidism is variable. In adrenal tumours, the reported short-axis size of the adrenal gland is more than 1 cm (more than 100% enlargement), ranging often between 2 and 4 cm²⁰⁻³². Additionally, adrenal tumours are mostly unilateral. Consequently, it is unlikely that an incorrect diagnosis of adrenal tumour would result from the increased adrenal gland size due to hyperthyroidism. However, cats with bilateral adrenal hyperplasia in pituitary-dependant hyperadrenocorticism, hyperaldosteronism or acromegaly have more variable adrenal gland size, from normal to mildly enlarged³³⁻³⁷. Hyperaldosteronism is diagnosed only in the late stage of adrenal adenoma or hyperplasia, as the associated increase in adrenal size can be small initially³⁸. Bilateral moderate adrenomegaly (5-10 mm in thickness) is described in 24% of cats with acromegaly³⁷. Consequently, moderate bilateral adrenal gland enlargement in hyperthyroid cats can be falsely associated with a suspicion of hyperadrenocorticism, hyperaldosteronism or acromegaly.

A potential limitation was the use of general anaesthesia for hyperthyroid cats whereas healthy cats were not anesthetized. It was decided to perform the ultrasonographic examination during anaesthesia for the pertechnetate scan in the hyperthyroid cats to decrease stress. The effect of propofol on adrenal gland metabolism has been studied in vitro or in humans but its effect on the adrenal gland size has not been assessed. Propofol had little to no effect on adrenocortical function in humans³⁹⁻⁴⁰. Propofol inhibited noradrenaline uptake and reduced catecholamine secretion by the adrenal medulla in

cultured bovine adrenal medullary cells, which may explain the propofol-induced hypotension during anaesthesia⁴¹. The effect of the propofol inhibition of the adrenal medulla on the adrenal size is unknown. However it is unlikely that this effect, limited to the short duration of the anaesthesia, creates a significant change in the general shape or size of the adrenal gland.

Another limitation is that adrenal disease could not be excluded completely in the hyperthyroid cats. However, as no cats had specific signs of adrenal disease at the time of the ultrasonographic examination, nor 4 to 6 months later, it is unlikely that the hyperthyroid cats had concurrent adrenal disease.

In conclusion, hyperthyroid cats have larger (20%) adrenal glands compared to healthy cats of similar age, which can be explained by the stimulation of the hypothalamic-pituitary-adrenocortical axis by hyperthyroidism. This can be misleading when a hyperthyroid cat is evaluated for adrenal disease or when hyperthyroidism and adrenal disease are both suspected initially. As adrenal tumours are obviously larger, the increase in the ultrasonographic size of the adrenal glands is not an issue in this instance. However if bilateral adrenal hyperplasia is suspected, hyperthyroidism should be considered as an alternative differential diagnosis to hyperadrenocorticism, hyperaldosteronism and acromegaly. Thus, total serum thyroxine concentration should be assessed in cases of bilateral ultrasonographic adrenomegaly.

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6 EFFECT OF PROPOFOL ANAESTHESIA ON ULTRASONOGRAPHIC MEASUREMENTS OF THE ADRENAL GLANDS IN HEALTHY CATS

Although real-time ultrasonography is feasible in awake cats, cats are anaesthetized for ultrasonography if they are uncooperative or during perioperative ultrasonographic examinations. Propofol induces hypovolemia in cats and potentially a venous vasodilation that redistributes blood volume in abdominal organs and may create organomegaly. Ultrasonographic adrenal measurements are compared before and during intravenous propofol anaesthesia in healthy cats. No significant effect of propofol anaesthesia on adrenal size is found, and the observed differences are small and clinically irrelevant. Compared to previous studies on pathological changes in adrenal size, the effect of propofol should not prevent ultrasonographic diagnosis of adrenomegaly.

Adapted from: Combes A., Vandermeulen E., Duchateau L., Peremans K., Hesta M., Saunders J.H. **Effect of propofol anaesthesia on ultrasonographic measurements of the adrenal glands in healthy cats.** Submitted in *Anatomia, Histologia, Embryologia*

6.1 INTRODUCTION

Ultrasonography of the feline adrenal glands was previously described in healthy and sick cats, either with or without sedation or anaesthesia (Table 14)¹⁻⁶. Ultrasonographic changes in the adrenal glands are also reported in hyperaldosteronism, hyperadrenocorticism, sex-hormone-producing tumour and phaeochromocytoma in cats⁷.

With new real-time high-definition ultrasound devices and increased experience of most of the ultrasonographers, the ultrasonographic examination of the feline adrenal gland is nowadays a quick procedure, feasible on awake cats in the majority of the cases². Nevertheless during perioperative examinations or with some aggressive cats, ultrasound must be performed under sedation or anaesthesia. However, anaesthetics often induce cardiovascular changes and some create morphological changes that can be detected by imaging. For instance, propofol has been demonstrated with computed tomography to induce splenomegaly within 10 minutes after intravenous administration while this was not observed with ultrasonography^{8,9}. Systemic hypotension and abdominal venous vasodilation are detected by direct pressure measurements in dogs anesthetized with propofol¹⁰. Propofol-induced hypotension is also described in cats¹¹. The redistribution of the blood volume within abdominal organs could induce organomegaly⁸.

The aim of this study is to evaluate whether morphological changes in the adrenal glands after an intravenous propofol bolus in healthy cats are observed with ultrasonography and whether it can be diagnostically relevant. This study is considered as a preliminary study in a research project about feline adrenal ultrasound in healthy and sick cats.

US measurements in mm		Healthy cats (n=94)*	Healthy anaesthetized cats (n=10)#	Healthy cats (n=20)§	Sick cats without endocrinopathies (n=24)@	Chronically sick cats (n=51)*	Diabetic cats (n=20)Φ
		mean± SD median (range)	mean± SD	median (range)	mean± SD median (range)	mean± SD median (range)	median (range)
LAG	Length	10.4 ± 1.8 10.5 (5.8-14.1)	10.7 ± 0.4	8.9 (4.5-13.3)	11.3 ± 2.8 10.6 (7.1-19.5)	10.8 ± 2.0 10.7 (6.9-15.5)	9.8 (7.1-11.3)
	Cranial height	3.8 ± 0.8 3.8 (1.8-5.9)	4.3 ± 0.3	3.9 (3.0-5.3)	3.8 ± 0.8 3.6 (2.8-4.7)	4.2 ± 0.9 4.3 (2.3-5.8)	3.5 (2.4-4.6)
	Caudal height	3.6 ± 0.7 3.5 (2.3-5.5)				3.9 ± 0.8 3.9 (2.0-6.1)	
RAG	Length	10.8 ± 1.9 10.8 (6.1-17.7)	10.7 ± 0.4	9.8 (6.7-13.7)	9.8 ± 2.4 9.9 (5.4-13.7)	10.9 ± 2.0 11.0 (6.7-14.7)	10.1 (4.6-13.2)
	Cranial height	3.7 ± 0.9 3.7 (1.5-6.7)	4.3 ± 0.3	3.9 (2.9-4.5)	4.5 ± 1.0 4.2 (3.4-7.1)	4.0 ± 1.2 4.1 (1.5-7.5)	3.6 (2.6-4.8)
	Caudal height	3.6 ± 0.7 3.7 (2.4-5.3)				3.9 ± 1.0 4.0 (1.6-6)	

TABLE 14 ULTRASONOGRAPHIC MEASUREMENTS OF THE ADRENAL GLANDS IN HEALTHY AND CHRONICALLY SICK CATS.

DATA FROM *COMBES ET AL., 2013, # CARTEE ET AL., 1993, § ZIMMER ET AL., 2000, @ ZATELLI ET AL., 2007, Φ KLEY ET AL., 2007.

6.2 MATERIAL AND METHODS

The study population consisted of 20 purpose-bred, group-housed cats. The cats were considered healthy based on a physical examination, urinalysis (specific gravity and dipstick), complete blood count, serum biochemistry, including serum potassium concentration and pre-anaesthetic systemic blood pressure measurement.

The study protocol was approved by the Ethical Committee of the Veterinary Faculty of the Ghent University (n° EC2012/190).

The anaesthetic protocol included an intravenous injection of propofol (Propovet Multidose 10 mg/ml, Abbott Laboratories Ltd, Maidenhead, UK), starting with a 2-ml bolus and continuing on demand. The total dose of propofol per cat needed was recorded.

Ultrasonographic examination of the adrenal glands was performed before and 15 minutes after anaesthesia induction, with a multifrequency linear transducer set on 13 MHz (CX-50, Philips Medical Systems, Brussels, Belgium). The abdomen was clipped before anaesthesia. Both pre- and post-anaesthetic examinations were performed with the cat in dorsal recumbency and coupling gel was applied.

The adrenal glands were imaged according to a previously described protocol^{2,5}. The maximum length (cranio-caudal, L) and the height of the cranial (crH) and caudal (cdH) poles (dorso-ventral) were measured on a sagittal scan of each adrenal gland. The diameter of the abdominal aorta at the level of the adrenal glands was also measured in a longitudinal axis. All the measurements were obtained during the examination by a single ultrasonographer. Additionally, all the images were recorded and remotely reviewed by the first author.

The ultrasonographic adrenal measurement before and during anaesthesia was compared using a paired t-test with cat as blocking factor. In a second analysis, it is investigated whether the within cat difference between pre- and per-anaesthetic measurements is influenced by the different continuous covariates (pre-anaesthetic systolic blood pressure, age, propofol dose) using a t-test. The influence of gender on this difference was studied by the Fischer's exact test. Finally, the relationship between the difference in adrenal measurements and the difference in aortic diameters, during and before anaesthesia, were investigated using Pearson correlation coefficients.

6.3 RESULTS

The 20 cats were all Domestic Shorthair, including 3 entire males, 4 castrated males, 10 entire females and 3 neutered females. Mean age with standard deviation (SD) was 4.4 ± 2.4 years. Mean weight with SD were 3.7 ± 1.0 kg. Mean pre-anaesthetic systolic blood pressure was 118 mmHg (SD = 12 mmHg). No relevant haematological or biochemical abnormalities were detected in urine or blood analysis.

Injected volume of propofol necessary for the anaesthesia ranged from 2,5 to 6 ml (median 4 ml), corresponding to a dose of 8 to 18.8 mg/kg (median 10 mg/kg).

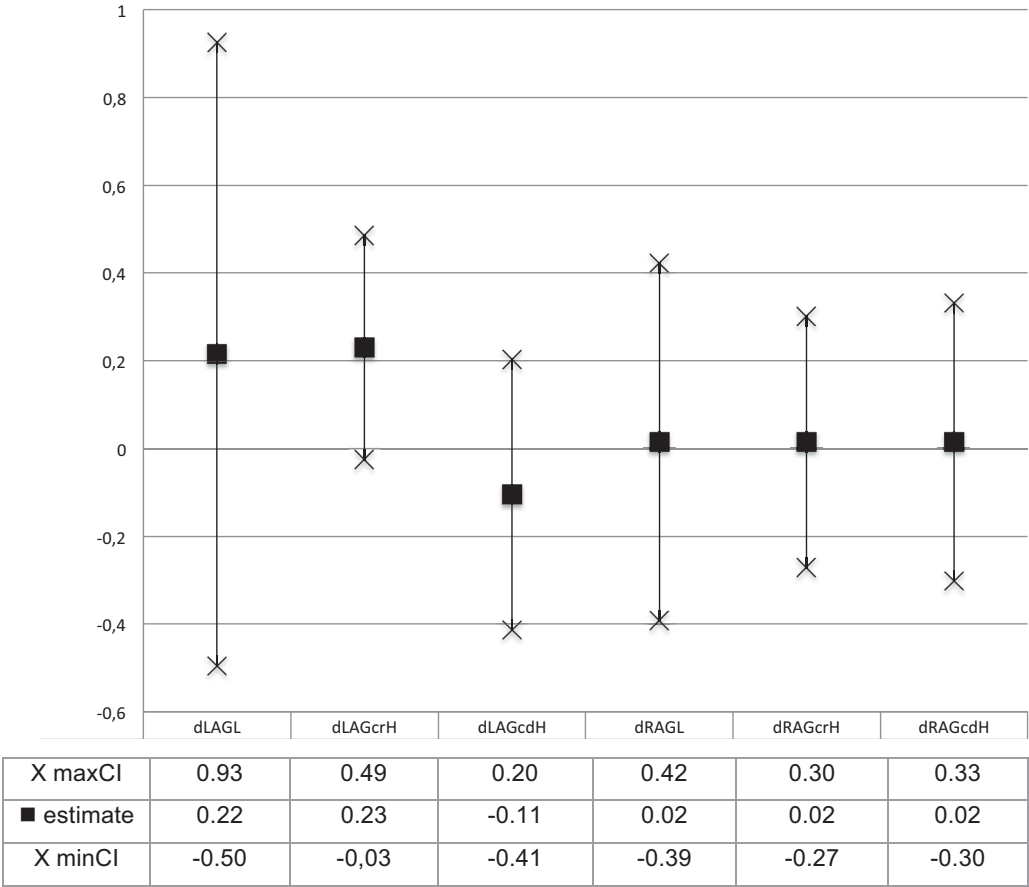
Ultrasonographic adrenal measurements before and during the anaesthesia are summarized in Table 15. No significant differences were found between pre- and per-anaesthetic measurements ($P > 0.05$).

US measurements in mm		Before propofol (mean +/- SD)	After propofol (mean +/- SD)	Difference (mean +/- SE)	P-value
LAG	Length	9.7 +/- 1.7	9.9 +/- 1.5	0.22 +/- 0.36	0.5603
	Cranial height	3.5 +/- 0.9	3.7 +/- 0.8	0.23 +/- 0.13	0.0934
	Caudal height	3.8 +/- 0.6	3.7 +/- 0.9	-0.11 +/- 0.16	0.5122
RAG	Length	10.8 +/- 1.9	10.8 +/- 1.7	0.02 +/- 0.21	0.9432
	Cranial height	3.9 +/- 0.8	3.9 +/- 0.7	0.02 +/- 0.15	0.9195
	Caudal height	3.9 +/- 0.7	3.9 +/- 0.8	0.02 +/- 0.16	0.9270
Aortic diameter		4.5 +/- 0.6	4.3 +/- 0.5	-0.18 +/- 0.12	0.1769

TABLE 15 ULTRASONOGRAPHIC MEASUREMENTS OF THE ADRENAL GLANDS AND THE AORTIC DIAMETER BEFORE AND DURING PROPOFOL ANESTHESIA. THE DIFFERENCE IN ULTRASONOGRAPHIC ADRENAL MEASUREMENT BEFORE AND DURING ANESTHESIA WAS PERFORMED USING A PAIRED T-TEST WITH CAT AS BLOCKING FACTOR.

The estimated difference before and during propofol anaesthesia and its 95% confidence interval are presented in Figure 20 for the different ultrasonographic adrenal measurements.

FIGURE 20 ESTIMATE AND 95% CONFIDENCE INTERVAL OF THE DIFFERENCE IN ULTRASONOGRAPHIC ADRENAL MEASUREMENTS BEFORE AND DURING PROPOFOL ANESTHESIA IN CATS (IN MM). dLAGL: DIFFERENCE IN LENGTH OF LEFT AG, dLAGcrH: DIFFERENCE IN CRANIAL HEIGHT OF LEFT AG, dLAGcdH: DIFFERENCE IN CAUDAL HEIGHT OF LEFT AG, dRAGL: DIFFERENCE IN LENGTH OF RIGHT AG, dRAGcrH: DIFFERENCE IN CRANIAL HEIGHT OF RIGHT AG, dRAGcdH: DIFFERENCE IN CAUDAL HEIGHT OF RIGHT AG.



No statistically significant effect of gender, age, blood pressure and propofol dose on the difference between the ultrasonographic adrenal gland measurements before and during anaesthesia, were detected.

Difference in aortic diameter was not correlated to the difference in adrenal measurements when comparing before and during propofol anaesthesia (all Pearson coefficients < 0.39).

6.4 DISCUSSION

No significant difference in ultrasonographic adrenal size was detected between pre-anaesthetic and per-anaesthetic measurements when using intravenous propofol. Similarly, the adrenal ultrasonographic measurements reported in a previous study on anesthetized cats showed comparable measurements as those in awake cats, although the cats were anesthetized with atropine and pentobarbital¹.

The observed differences between pre-anaesthetic and per-anaesthetic measurements when using intravenous propofol were small and clinically irrelevant. Based on their 95% confidence intervals, it can be concluded that the difference for left adrenal length is contained in the interval $[-0.50;0.93]$, for left cranial adrenal height in the interval $[-0.03;0.49]$ and for left caudal adrenal height in the interval $[-0.41;0.20]$. The differences for the right gland were even smaller.

Comparing to the intensity of the changes in adrenal size reported in studies about adrenal diseases, the effect of propofol should not prevent ultrasonographic diagnosis of adrenomegaly. For instance, in hyperthyroid cats, the adrenal glands show similar or mildly enlarged measurements with mean measurement differences of 1.6-1.7 mm in length and 0.8-0.9 mm in height⁶. In cats with hyperaldosteronism secondary to an adrenal carcinoma, the adrenal mass is reported as at least twice the normal adrenal size on ultrasound, or more than 10 and 3 mm difference in length and height measurements, respectively⁵.

Enlargement of any abdominal organ during propofol anaesthesia could be expected based upon the cardiovascular effect of propofol. Redistribution of

blood in the abdominal venous circulation is described secondary to propofol-induced hypotension and venous vasodilation in dogs⁸. Propofol increases systemic vascular capacitance as a result of an inhibition of sympathetic nervous system in rats¹². Propofol-induced hypotension was studied in vitro and a smooth muscle relaxation was induced by propofol with a lower dose in the vein than in the artery¹³. These findings are hypothesized by the authors in a computed tomography study of propofol-induced splenomegaly in dogs⁸. Even though the adrenal glands are richly vascularized and contain large cortical arterial and medullary venous sinuses, the potential smooth muscle relaxation and vasodilation induced by propofol may not be sufficient to change significantly the ultrasonographic size of the glands¹⁴. Although propofol-induced systemic hypotension is also described in cats, the blood redistribution hypothesis is not studied in this species¹¹.

During the present study, performing adrenal ultrasound in an anaesthetized cat appeared easier than in an awake cat for different reasons. Abdominal muscle relaxation allowed a better skin contact and a better pressure from the transducer. An immobile animal with slower breathing pattern provided a better image quality and a more comfortable examination for the ultrasonographer. Adrenal glands appeared easier to find and better delineated, which may allow more accurate measurements under anaesthesia. Pre-anaesthetic measurements may be impeded by breathing or motion thus under- or over-estimating the real adrenal size and creating a difference in adrenal measurements between pre-anaesthetic and per-anaesthetic examination. The accuracy of the measurements was not tested however in this study, as we could not compare ultrasonographic measurements to a gold standard reference such as gross measurements, for obvious ethical reasons. In conclusion, no change in ultrasonographic adrenal measurements secondary to intravenous propofol anaesthesia in cats could be detected. Intravenous propofol is not likely to influence ultrasonographic diagnosis of adrenal

diseases. Propofol can be used in uncooperative cats or in perioperative cases for evaluation of the feline adrenals without effect on the morphological appearance of the adrenal glands.

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7 GENERAL DISCUSSION

Diseases of the adrenal glands are rare in cats but do occur, for example cases of primary hyperaldosteronism, pituitary- or adrenal-dependant hyperadrenocorticism, sex hormone-secreting neoplasia, pheochromocytoma and other adrenal neoplasms are reported. However, probably because of their scarcity, there is no prospective study on the imaging findings of these adrenal diseases in the literature but only descriptions in case reports and textbooks¹⁻³⁸. Ultrasonography (US) is the most commonly used imaging modality in those case reports and to assess the abdominal organs in cats in general. US is non-expensive, non-invasive and nowadays widely available.

The ultrasonographic appearance of the feline adrenal gland is reported to be poorly reliable for diagnosis of adrenal diseases such as adrenal hyperplasia in pituitary-dependant hyperadrenocorticism or primary hyperaldosteronism^{2,31}. The morphological changes of the adrenal gland may be too inconspicuous to be detected. Additionally, the reference ultrasonographic scanning technique, images and sizes of the feline adrenal glands are poorly described in the literature and they are not standardized³⁹⁻⁴¹. This may explain for part of the unreliability of this modality to diagnose adrenal diseases in cats. However, recent improvements in knowledge and skills of the ultrasonographers and US material allow a more systematic assessment of the adrenal glands in any abdominal ultrasound examination.

The general aim of this thesis was to optimize the protocol for US examination of the feline adrenal glands and to describe the US appearance of these glands in disorders that may affect their appearance.

The first aim of this thesis (chapter 3) was to create a standardized protocol for the ultrasonographic examination of the adrenal glands in cats, in order to compare the normal and pathological appearance of the glands more reliably. The ultrasonographic protocol must be easy to understand and perform,

repeatable, with a low inter- and intra-observer variability and a low inter- and intra-individual variability.

The aim was to test descriptive criteria and several anatomical measurements of the adrenal gland. Although the classical criteria (shape, location, outline, echogenicity, size) are theoretically applied to the ultrasonographic description of the adrenal glands, some appear too subjective. Indeed, different observers do not describe the shape of the gland in the same way in this study. On the other hand, the echogenicity, the layering pattern and the presence of hyperechoic foci show a better intra- and inter-observer agreement. Therefore, they can be used as reliable descriptive criteria of the feline adrenal glands. The description may also be improved by comparing their echogenicity with that of the neighbouring hepatic parenchyma or by reporting the outline of the gland.

The size of the adrenal glands can be determined in one, two or three dimensions, through linear, area or volumetric measurements, respectively. Area and volume measurements are not assessed with B-mode ultrasound as they are calculated from the linear measurements and thus, subjected to a larger error (total error = multiplication of the error of each linear measurement). Adrenal size determination is therefore limited to linear measurements. Each linear measurement should be defined by a scan plane and an anatomical distance. Although the length is clearly defined anatomically as the craniocaudal distance, the short axis of a flattened, ovoid organ such as the feline adrenal glands is more difficult to define precisely. In this study, the cats were scanned in dorsal recumbency, from a ventral window, in order to limit the scan planes to sagittal and transverse planes of the gland. Then, the dorsoventral distance is called the height and the lateromedial distance the width. The adrenal measurements obtained in the present study are comparable to a previous study in ten healthy cats³⁹. Except in this latter study on ten cats, width, height, thickness and diameter are often compared in the literature without clear anatomical definition. As the feline adrenal gland is

ovoid and flattened dorsoventrally, the difference between the height and the width may be significant.

Two studies determined the difference between *in vivo* ultrasonographic measurements and post-mortem gross measurements of the adrenal glands in ten cats^{39,40}. The ultrasonographic measurements of the width in a transverse plane were underestimated compared to the gross measurement (about 2 mm) while the length and the height measurements correlated well³⁹. In the present study, we also found the width to be more variable than the height measurements and the transverse plane measurements to be more variable than the sagittal plane measurements. Although it has not been evaluated here, the variability of the width measurement may be decreased thanks to a dorsal scanning plane from a lateral ultrasonographic window with the cat in lateral recumbency.

Interestingly, the cranial short-axis measurements of the right adrenal gland and the length measurements of the left adrenal gland were the most variable. This may be explained by the observation that those measurements are technically more challenging, because of a close contact of the right adrenal gland with the liver and an increased left kidney and adrenal mobility.

The optimized ultrasonographic protocol has been applied to healthy cats in chapter 4 and compared with pathological conditions. It consists of a sagittal scan of each adrenal gland with length and height measurements and assessment of the echogenicity, the layering and the presence of hyperechoic foci. The most common normal appearance of the feline adrenal gland is a well-delineated, bilobed, hypoechoic structure, surrounded by a hyperechoic fat halo.

A few healthy cats showed variable hyperechoic spots, however it is more common in sick cats. No distal acoustic shadowing is noticed, which is confusing as those foci were previously described as mineralizations⁴². These

foci still may be mineralizations that are too small to create the artefact or they may represent fibrosis, haemorrhage or fat in the gland. Unfortunately, there is no histopathological analysis of these foci.

Reference ranges of ultrasonographic adrenal measurements have been published based on small populations of cats³⁹⁻⁴¹. In the present study, reference intervals of adrenal measurements were inferred from the values obtained in 145 cats without adrenal diseases. The results are similar to those of previous studies, although the upper limits are higher in the present population. Several technical issues may account for this difference. The length may be underestimated if the sagittal plane is not aligned with the gland axis but with the body axis⁴³. As already discussed, the short axis measurements are not precise enough while width and height may have been confused in previous studies, thus representing measurements of different anatomical distances^{40,41}. No difference in adrenal measurements between males and females was reported⁴⁰. Another study did not find any significant correlation between the bodyweight, the body surface or the body condition score and adrenal measurements⁴¹. In the present population, an adrenal enlargement has been detected in castrated cats (combined males and females) and a shortening of the adrenal length was also noticed with aging, explaining the clinical impression of rounding of the gland in older cats. However, these statistically significant effects were mild and not clinically relevant. In chapter 5, no significant effect of gender, age, breed or weight was found in the control healthy cats.

This ultrasonographic protocol can then be applied to cats with adrenal diseases, to determine the changes in the descriptive and quantitative criteria and to assess the accuracy of ultrasonography to diagnose adrenal diseases in cats. Unfortunately, the rare occurrence of adrenal diseases in cats makes a prospective study difficult to achieve. Ultrasonographic data about cats with

adrenal diseases were therefore collected retrospectively. Inclusion criteria were based on a definitive diagnosis of the adrenal disease and good quality ultrasonographic images and reports, allowing the remote, retrospective assessment of all the criteria from the protocol established in chapter 3. However, the main limitation of this comparison in chapter 4 is the variability between institutions, devices, observers and time. For instance, an inaccurate scan plane cannot be excluded based on fixed images; changes in outline or echogenicity of the gland outside the image plane are not assessed.

With the strict inclusion criteria, only six cats with primary hyperaldosteronism and four cats with pituitary-dependant hyperadrenocorticism were found in the database of two academic institutions over eleven years (2000-2011). Regarding the cats with hyperaldosteronism, the main ultrasonographic finding was a unilateral mass, clearly larger than the reference interval of adrenal measurements in healthy cats. The descriptive features of the mass do not allow differentiation between benign and malignant adrenal lesions. Local or vascular invasion is not detected, and thus does not help in this differentiation. Two cases, initially diagnosed as unilateral adrenal mass, were later confirmed as bilateral carcinoma after necropsy. This ultrasonographic misdiagnosis has already been reported in a bilateral adenoma³². Additionally, two cases of bilateral adrenal hyperplasia did not show ultrasonographic abnormalities³¹. Those atypical cases enhance a major limitation of ultrasound in the diagnosis of primary hyperaldosteronism, meaning that a normal contralateral gland to an adrenal mass or bilateral normal adrenal glands do not preclude benign or malignant adrenal infiltration. This statement is highly relevant if unilateral adrenalectomy is the planned treatment. Moreover, the occurrence of bilateral adrenal infiltration is most likely underestimated as the contralateral gland was rarely analysed histopathologically in the reported cases.

Considering the four cats with pituitary-dependant hyperadrenocorticism, the present study and the previously reported cases pointed out the limited value of ultrasound in diagnosing feline adrenal hyperplasia²⁻¹⁷. Most cases showed mild bilateral, symmetrical enlargement of the adrenal glands or measurements in the upper range of the reference intervals. Interestingly, all cats of this study showed hyperechoic foci in the adrenal parenchyma. Although it cannot be tested here without cases of adrenal tumours, the ultrasonographic measurements of the adrenal glands may determine the adrenal symmetry or asymmetry, thus orientate toward a pituitary or adrenal origin of the hyperadrenocorticism. For instance, in dogs, the adrenal asymmetry has been quantified with ultrasound and computed tomography, depending on the origin of the hyperadrenocorticism^{44,45}. However, in atypical cases, such as bilateral adrenal tumours or asymmetrical adrenal hyperplasia, ultrasonographic findings may remain misleading.

Improving the ultrasonographic diagnosis of adrenal diseases in cats is first limited by technical issues. The standardization of the least variable ultrasonographic protocol manages to minimize those issues (Chapter 3). The inherent poor morphological changes of the adrenal gland in some diseases also limit the value of ultrasound for their diagnosis. Advantages and drawbacks of ultrasound in adrenal diseases are listed in chapter 4. Confusing factors such as acute or chronic stress, concurrent diseases, medication or anaesthesia may additionally disturb ultrasonographic diagnosis of adrenal diseases. The effect of chronic disease, hyperthyroidism and propofol anaesthesia are studied in chapter 4 to 6, respectively.

In chapter 4, the ultrasonographic features of the adrenal glands in cats with various chronic diseases are not significantly different from those in healthy cats. Only the hyperechoic foci are more frequent in sick cats compared to

healthy cats. Ultrasonographic measurements are similar in the two groups of cats, as previously hypothesized in different studies^{41,46}. This finding is extremely important, as the cats presented at the veterinary hospital for ultrasound examination are sick. Chronic diseases, stress or hospitalisation may create false positive results in some biological tests in cats, such as urine cortisol:creatinine ratio, low dose dexamethasone suppression test, ACTH stimulation test or plasma free metanephrines evaluation⁴⁶⁻⁵⁰. The present study rules out concurrent non-endocrine diseases as a confounding factor in the ultrasonographic diagnosis of adrenal diseases.

Nevertheless, some endocrine diseases are known to influence adrenal function and potentially adrenal morphology. For instance, acromegaly has already been described as a potential cause for bilateral adrenomegaly^{51,52}.

In chapter 5, hyperechoic foci are present more frequently in the adrenal glands of the hyperthyroid cats, as in cats with various chronic diseases or with pituitary-dependant hyperadrenocorticism (chapter 3). It is hypothesized to be linked to the chronic stimulation of the hypothalamic-pituitary-adrenal axis.

Hyperthyroidism is indeed associated with exaggerated responsiveness of the adrenal cortex in cats^{47,54,55}. ACTH stimulation of the adrenal cortex is enhanced and cortisol clearance is increased. Consequently, basal cortisol is on the contrary, rarely elevated. In a necropsy study, nodular adrenocortical hyperplasia was found in 8/23 cats with hyperthyroidism⁵³. The present morphological study describes indeed a mild, symmetrical adrenal enlargement in hyperthyroid cats. As in the necropsy study, it does not affect all hyperthyroid cats. Although there is an overlap with the reference interval, the upper range of ultrasonographic adrenal measurements is higher in hyperthyroid cats. Looking at another study in hyperthyroid cats, their ultrasonographic data show a similar overlap indeed, but also a higher mean and upper limit for adrenal width in hyperthyroid cats compared to sick cats⁵⁵. This adrenal enlargement represents an increase in 11 to 24% of the adrenal

measurements. Clinically, this may not interfere with the diagnosis of adrenal tumours. However, hyperthyroidism may be added in the differential diagnosis of mild, symmetrical, bilateral adrenomegaly, with hyperadrenocorticism, acromegaly and hyperaldosteronism. Therefore, the total serum thyroxin concentration may be advised in the biological work, if clinically indicated.

In chapter 5, the hyperthyroid cats were scanned under propofol anaesthesia for practical and ethical reasons, whereas the control cats were scanned without anaesthesia. Propofol (2,6-diisopropylphenol) is an intravenous anaesthetic, which is used both for induction and maintenance of general anaesthesia. Although the metabolic effect of propofol on the adrenal glands has been thoroughly explored, the morphological changes on the gland have not been assessed.

First, the effect of propofol on the cortical secretion in the adrenal glands is controversial^{56,57}. Although, propofol had a direct antisteroidogenic effect on adrenocortical cells *in vitro*; it did not inhibit significantly adrenocortical cortisol secretion in response to ACTH in humans⁵⁶.

Second, propofol commonly resulted in hypotension associated with decreases in cardiac output and systemic vascular resistance⁵⁸. These cardiovascular effects of propofol were determined by inhibition of the sympathetic system, especially in the adrenal medulla (modified postganglionic neurons which secrete epinephrine and norepinephrine directly into the blood stream rather than into a synapse)⁵⁹. The effect of propofol on the metabolism of the adrenal medulla has been studied *in vitro*, in animals and in humans. Propofol inhibited catecholamine secretion from the adrenal medulla in cultured bovine adrenal medullary cells. In the same study, serum noradrenaline and arterial blood pressure were lowered after intravenous injection of an anaesthetic dose of propofol in rats⁵⁸. These combined findings may explain the propofol-induced hypotension during anaesthesia. The effect of this adrenal inhibition of catecholamine secretion on the adrenal size is unknown. However, it was

unlikely that this metabolic effect, limited to the short duration of the anaesthesia, created a significant change in the general shape or size of the adrenal gland.

The potential morphological consequences of propofol anaesthesia on adrenal ultrasonographic appearance was studied in chapter 6: propofol did not show any significant effect in cats. First, as anaesthesia allowed a more comfortable examination, with better skin contact and relaxed abdominal muscles, a better image quality and measurement accuracy might be expected. However, according to this study in chapter 6, it did not interfere with the ultrasonographic diagnosis of adrenal diseases.

Second, propofol-induced hypotension has been reported in cats⁶⁰. It has been suggested that the hypotension may result from a direct vasodilator action on the veins and arterioles⁶¹. In a study on hepatic veins and aorta in rats, propofol caused a dose-related decrease in potassium-induced tone in arteries and veins⁶². This has been confirmed in an *in vivo* study in rats that showed the increase in vascular capacitance with especially dose-dependant venodilation secondary to propofol inhibition of sympathetic vasoconstrictor activity⁶³. As already described in the spleen, the propofol-induced venodilation may elicit organomegaly by enlargement of the vascular bed in a richly vascularized organ⁶⁴⁻⁶⁵. However, propofol-induced adrenomegaly was not detected with ultrasound in our study.

As future perspectives to improve adrenal ultrasound in cats, other factors that may influence the morphology of the adrenal glands, such as acute stress may be evaluated. A prospective study on a larger number of cats with adrenal diseases, including all types of hyperadrenocorticism, sex hormones-secreting tumours, acromegaly would be very interesting, although the scarcity of these diseases imply a long study time. Furthermore, contrast-enhanced ultrasound

may be applied to the diagnosis of adrenal diseases in cats, to highlight differences in perfusion and vascularity between normal glands, adrenal hyperplasia and benign or malignant adrenal tumours, as it has been recently reported in dogs^{66,67}.

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SUMMARY

Ultrasound is a routine imaging modality to assess abdominal organs in cats. Yet, the small size of the adrenal glands makes it challenging. Reference literature about ultrasonographic features of adrenal glands in healthy and sick cats is rare, sparsely distributed in multiple case reports and textbooks and difficult to apply in practice.

Detection of specific ultrasonographic abnormality at the level of the adrenal gland can guide the clinician into the optimal selection of endocrine blood tests or confirm a clinical or biological suspicion of adrenal disease. Ultrasonography of the adrenal glands is interesting for detection of adrenal asymmetry caused by a unilateral adrenal mass. Moreover, it allows detection of local invasion and abdominal metastases.

Adrenal ultrasonography may be improved by using a standardized protocol, with clear ultrasonographic criteria to define the reference range of normal adrenal glands compared to adrenal diseases, concurrent diseases or other confounding factors.

Interpretation of ultrasonographic measurements requires an understanding of the source and the magnitude of variation. A substantial part of the variation is often due to the observer, the equipment or the animal. In **chapter 3**, the aim of the study is to evaluate which adrenal gland measurement is the least variable within and between observers. Three experienced ultrasonographers examined 6 cats at 3 different times according to a strict scanning protocol. Seven ultrasonographic measurements were performed on each adrenal gland (maximal length, maximal height at the cranial and caudal poles on sagittal and transverse images, and maximal width of the cranial and caudal poles). Height measurements in both planes showed the lowest variability within and between observers compared to length and width measurements. Descriptive ultrasonographic features such as echogenicity of the gland, presence of hyperechoic spots or layering assessment demonstrated a satisfactory to good intra- and interobserver agreement whereas the shape assessment showed a

very poor interobserver agreement. These results provide a reliable scanning protocol to further evaluate the adrenal glands in cats with hyperaldosteronism, hyperadrenocorticism, a sex-hormone-producing tumour or other adrenal diseases.

In **chapter 4**, the first part of the study aimed to describe prospectively the ultrasonographic features of the adrenal glands in 94 healthy cats and 51 chronically sick cats. It confirmed the feasibility of ultrasonography of adrenal glands in healthy and chronically sick cats, which were not statistically different. The typical hypoechoic appearance of the gland surrounded by hyperechoic fat made it recognizable. A sagittal plane of the gland, not in line with the aorta, may be necessary to obtain the largest adrenal measurements. The reference intervals of adrenal measurements were inferred from the values obtained in the healthy and chronically sick cats (mean \pm 1.96*SD): the adrenal length was 6.9 to 14.4 mm; the cranial height was 2.0 to 5.7 mm; the caudal height was 2.2 to 5.2 mm. The second part of the study consisted in a retrospective analysis of the ultrasonographic examination of the adrenal glands in cats with adrenal diseases (6 hyperaldosteronism, 4 pituitary-dependent hyperadrenocorticism) and a descriptive comparison with the reference features obtained in the control groups from the prospective study. Cats with hyperaldosteronism presented with a unilateral severely enlarged adrenal gland. However, a normal contralateral gland did not preclude a contralateral infiltration in benign or malignant adrenal neoplasms. The ultrasonographic appearance of the adrenal glands could not distinguish benign from malignant lesions. The ultrasonographic appearance of pituitary-dependent hyperadrenocorticism was mainly a symmetrical adrenal enlargement, although a substantial number of cases were within the reference intervals of adrenal size.

Feline hyperthyroidism is potentially associated with exaggerated responsiveness of the adrenal gland cortex. In **chapter 5**, the adrenal glands of

23 hyperthyroid cats were examined ultrasonographically and compared to the adrenal glands of 30 control cats. Ten hyperthyroid cats had received antithyroid drugs until 2 weeks before sonography, the other 13 were untreated. There was no difference in adrenal gland shape between healthy and hyperthyroid cats: bean-shaped, well-defined, hypoechoic structures surrounded by a hyperechoic halo in 43/60 (71.6%) healthy cats and 34/46 (73.9%) hyperthyroid cats; more ovoid in 13/60 (21.6%) healthy cats and 9/46 (19.6%) hyperthyroid cats while more elongated in 4/60 (6.7%) healthy cats, 3/46 (6.5%) hyperthyroid cats. Hyperechoic foci were present in 9/23 (39.1%) hyperthyroid cats and 2/30 (6.7%) healthy cats. The adrenal glands were significantly larger in hyperthyroid cats, although there was overlap in size range. The mean difference between hyperthyroid cats and healthy cats was 1.6 and 1.7 mm in left and right adrenal gland length, 0.8 and 0.9 mm in left and right cranial adrenal gland height and 0.4 and 0.9 mm in left and right caudal adrenal gland height. There was no significant difference between the adrenal gland measurements in treated and untreated hyperthyroid cats. The adrenomegaly was most likely associated with the hypersecretion of the adrenal cortex documented in hyperthyroid cats. Hyperthyroidism should be a alternative differential diagnosis to hyperadrenocorticism, hyperaldosteronism and acromegaly in cats with bilateral moderate adrenomegaly.

Although real-time ultrasonography is feasible in awake cats, cats are anaesthetized for ultrasonography if they are uncooperative or during perioperative ultrasonographic examinations. Propofol induces hypovolemia in cats and potentially a venous vasodilation that redistributes blood volume in abdominal organs, thus creating organomegaly. In **chapter 6**, ultrasonographic adrenal measurements were compared before and during intravenous propofol anaesthesia in healthy cats. No significant effect of propofol anaesthesia on adrenal size was found, and the observed differences are small and clinically irrelevant. Compared to previous studies on pathological changes in adrenal

size, the effect of propofol should not prevent ultrasonographic diagnosis of adrenomegaly.

SAMENVATTING

Echografie is een courante beeldvormingstechniek voor de beoordeling van abdominale organen bij de kat. Toch maken de kleine afmetingen van de bijnieren echografische beoordeling moeilijk. Referentieliteratuur over echografische kenmerken van de bijnieren bij gezonde en zieke katten is zeldzaam, en verspreid over verschillende gevalsstudies en handboeken en zodanig moeilijk toe te passen in de praktijk.

Detectie van echografische abnormaliteiten in de bijnier kan van invloed zijn op de keuze van endocriene bloedtesten of kan een bevestiging betekenen van een klinisch of biologisch vermoeden van een bijnieraandoening.

Echografie van de bijnieren is eveneens interessant voor de detectie van bijnierasymmetrie veroorzaakt door een unilaterale bijniermassa. Bovendien laat het toe te screenen op lokale invasie en abdominale metastasen.

Bijnier echografie kan ondersteund worden door het gebruik van een gestandaardiseerd protocol, met duidelijke echografische criteria om de referentiewaarden van de normale bijnier te vergelijken met dat van bijnierziekten, geassocieerde ziekten en andere verwarrende factoren.

Interpretatie van echografische metingen vereist kennis van de oorsprong en de grootte van mogelijke variaties. Een aanzienlijk deel van deze variatie kan toegeschreven worden aan de beoordelaar, het gebruikte toestel of het dier.

In **hoofdstuk 3** is het doel van de beschreven studie te evalueren welke bijniermeting het minst varieert over verschillende beoordelaars. Drie ervaren radiologen onderzochten 6 katten op 3 verschillende tijdstippen volgens een strikt scanprotocol. Echografische metingen werden uitgevoerd op elke bijnier (maximale lengte, maximale hoogte op de craniale en caudale polen op sagittale en transverse beelden, en maximale breedte aan de craniale en caudale polen). Hoogtemetingen in beide vlakken vertoonden de laagste intra- en interbeoordelaarsvariabiliteit in vergelijking met lengte- en breedtemetingen.

Beschrijvende echografische kenmerken zoals de echogeniciteit van de bijnier, de aanwezigheid van hyperechogene zones of de beoordeling van lagen bleek voldoende intra- en interbeoordelaarsovereenstemming te vertonen, terwijl de beoordeling van de vorm het tegengestelde vertoonde. Deze resultaten helpen bij het opstellen van een betrouwbaar scanprotocol om verder de bijnieren te beoordelen van katten met hyperaldosteronisme, hyperadrenocorticisme, geslachtshormonen-producerende tumor of andere bijnieraandoeningen.

In **hoofdstuk 4**, beschrijft het eerste deel van de studie de echografische kenmerken van de bijnieren bij 94 gezonde katten en 51 chronisch zieke katten. Dit bevestigt de haalbaarheid van echografie van de bijnieren bij gezonde en zieke katten, die statistisch geen significant verschil toonde. Het typisch hypoëchogene voorkomen van de bijnier omgeven door hyperechogeen vet zorgt voor een snelle identificatie. Een sagittale opname van de bijnier, niet in lijn met de aorta, kan noodzakelijk blijken om de grootste bijniermetingen uit te voeren. De referentie-intervallen bij bijniermetingen werden afgeleid van waarden bekomen bij gezonde en chronisch zieke katten (gemiddelde $\pm 1.96 \times \text{SD}$): de bijnierlengte was 6.9 tot 14.4 mm; de craniale hoogte was 2.0 tot 5.7 mm; de caudale hoogte was 2.2 tot 5.2 mm. Het tweede deel van de studie bestond uit een retrospectieve analyse van een echografisch onderzoek van de bijnieren bij katten met een bijnierziekte. (6 hyperaldosteronisme, 4 hypofyse-afhankelijk hyperadrenocorticisme) en een beschrijvende vergelijking met referentiewaarden verkregen bij controlegroepen uit de prospectieve studie. Katten met hyperaldosteronisme boden zich aan met een unilateraal ernstig vergrote bijnier. Desalniettemin sloot een normale contralaterale bijnier geen mogelijke contralaterale infiltratie uit met goedaardige of kwaadaardige bijnierneoplasie. Het echografisch voorkomen van de bijnieren kan niet gebruikt worden bij de differentiatie van goedaardige of kwaadaardige lesies. Het echografische voorkomen van hypofyse-afhankelijk hyperadrenocorticisme was vooral een symmetrische vergroting van de bijnieren, maar bij een

aanzienlijk deel van de gevallen bleef de grootte van de bijnier binnen het referentie-interval.

Feliene hyperthyroidie is mogelijk geassocieerd met een overdreven respons van de bijniercortex. In **hoofdstuk 5** werden de bijnieren van 23 hyperthyroïde katten echografisch onderzocht en vergeleken met die van 30 controlekatten. Tien hyperthyroïde katten kregen antithyroïde medicatie tot 2 weken voor de echografie, de 13 overige waren onbehandeld. Er was geen verschil tussen de bijnervorm bij gezonde en hyperthyroïde katten: boonvormige, goed gedefinieerde, hyoëchogene structuren omringd door een hyperechogene halo bij 43/60 (71.6%) gezonde katten en 34/46 (73.9%) hyperthyroïde katten; eerder ovaal bij 13/60 (21.6%) gezonde katten en 9/46 (19.6%) hyperthyroïde katten en, eerder langwerpig bij 4/60 (6.7%) gezonde katten en 3/46 (6.5%) hyperthyroïde katten. Hyperechoische foci waren aanwezig bij 9/23 (39.1%) hyperthyroïde katten en 2/30 (6.7%) gezonde katten. De bijnieren waren significant groter bij hyperthyroïde katten hoewel er een overlap was in grootte. Het gemiddelde verschil tussen hyperthyroïde en gezonde katten was 1.6 and 1.7 mm tussen linker- en rechterbijnierlengte, 0.8 and 0.9 mm tussen craniale linker- en rechterbijnierhoogte en, 0.4 and 0.9 mm tussen caudale linker- en rechterbijnierhoogte. Er was geen significant verschil tussen de bijniermetingen bij behandelde en niet-behandelde katten. Adrenomegalie was meestal geassocieerd met de hypersecretie van de bijniercortex bij hyperthyroïde katten. Hyperthyroidie zou een alternatieve differentiaal diagnose kunnen zijn voor hyperadrenocorticisme, hyperaldosteronisme en acromegalie bij katten met bilaterale matige adrenomegalie.

Hoewel B-mode echografie haalbaar is bij katten die wakker zijn, worden katten gesedeerd indien ze onhandelbaar zijn of bij perioperatieve echografische onderzoeken. Propofol veroorzaakt hypovolemie bij katten en mogelijk ook veneuze dilatatie die het bloedvolume herverdeeld naar abdominale organen. Dit kan organomegalie veroorzaken. In **hoofdstuk 6**

werden echografische bijniermetingen vergeleken voor en tijdens intraveneuze propofolanesthesie bij gezonde katten. Geen significant effect werd gevonden van de propofolanesthesie op de bijniergrootte, en de geobserveerde verschillen waren klein en klinisch irrelevant. Het gebruik van propofol heeft geen negatief effect op de echografische diagnose van adrenomegalie.

CURRICULUM VITAE

Anaïs Combes was born on January 29th 1983 in Sens (France). After gaining the “Baccalaureat” in the Lycée Louis-le-Grand in Paris, she studied at the National Veterinary School of Toulouse (France) from 2001 to 2005. She then spent an optional fifth year of veterinary studies in small animals medicine and surgery at the Veterinary faculty of St Hyacinthe, University of Montréal (Canada), followed by a rotating internship in small animals medicine and surgery at the National Veterinary School of Maisons-Alfort (France). She graduated as a veterinary surgeon in June 2007 by presenting her thesis in veterinary medicine entitled “Contribution à l’imagerie médicale de *Macropus rufogriseus*”.

Anaïs Combes spent 2 years in the small animals clinic of the National Veterinary School of Maisons-Alfort as a clinical assistant in internal medicine and diagnostic imaging, before joining Ghent University in 2009 to complete a 4-year residency in medical imaging. She became a Diplomate of the European College of Veterinary Diagnostic Imaging in 2013. She finished her PhD project on ultrasonography of the feline adrenal gland and is currently working in the referral practice Alliance in Bordeaux.

Anaïs Combes is the author or co-author of 13 scientific publications in international veterinary journals and 4 scientific publications in Belgian and French veterinary journals. She also took part actively in several national and international congresses.

International peer-reviewed journals

- ▶ **A. COMBES**, E. VANDERMEULEN, L. DUCHATEAU, K. PEREMANS, M. HESTA, J.H. SAUNDERS. Effect of propofol anaesthesia on ultrasonographic measurements of the adrenal glands in healthy cats. *Anat Histol Embryol* (submitted to *Anatomia, Histologia, Embriologia*).
- ▶ **A. COMBES**, E. STOCK, E. VAN DER VEKEN, L. DUCHATEAU, B. VAN RYSEN, J.H. SAUNDERS. Ultrasonographic examination of feline adrenal glands: intra- and interobserver variability. *J Feline Med Surg* (accepted).
- ▶ **A. COMBES**, J.H. SAUNDERS. Ultrasonographic examination of the feline adrenal glands: a review. *Pratique médicale et chirurgicale de l'animal de compagnie* (accepted for publication in 2014).
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- ▶ **A. COMBES, P. PEY, I. PUTCUYPS, A. BEDU, L. DUCHATEAU, P. DE FORNEL-THIBAUD, G. BENCHEKROUN, D. ROSENBERG, S. DAMINET, J. SAUNDERS.** Echographie des glandes surrénales dans 4 populations de chats : sains, malades chroniques, atteints d'hyperaldostéronisme et atteints d'hypercorticisme d'origine pituitaire. Tribune résidents et internes du congrès AFVAC-AVEF, 2012, Paris (France).
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- ▶ **A. COMBES, EVA VANDERMEULEN, LUC DUCHATEAU, KATHELIJNE PEREMANS, SYLVIE DAMINET, JIMMY SAUNDERS.** Ultrasonographic measurements of adrenal glands in cats with hyperthyroidism. Présentation orale au congrès EVDI, 2011, London (UK). Best resident presentation Award.
- ▶ **A. COMBES, T. DE CHALUS, A.S. LEPELIER, P. PEY, SYLVIE DAMINET, LUC DUCHATEAU, JIMMY SAUNDERS.** Ultrasonographic adrenal gland measurements

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ACKNOWLEDGMENTS

First of all, I would like to thank my supervisor and promotor, Jimmy Saunders, for these 4 years of advice, support and guidance in veterinary medical imaging. I discovered the clinical research, as a teamwork and as a personal work. These 4 years made me more curious, structured, critical and persevering. Although I explored the field of veterinary imaging in my residency, my PhD allowed me to find out the limits of scientific knowledge and the desire to know more, to do better and to improve my everyday work. Thank you for all the professional, clinical, scientific, political and personal guidance and exchange during these 4 years. I hope we could keep on exchanging!

I would like to thank the chairman of the examination committee, Pr Piet Deprez, for accepting to lead the PhD revision and defense.

I would like to thank Sofie Bhatti and Catherine Delesalle for accepting to be in the examination committee and for their constructive advice in the revision of this PhD.

A special thank word for Eva Vandermeulen: thank you for accepting to be in the examination committee and for all your help and support in all the studies of this work. Working with you in research and in the clinic was a pleasure. You are so great to explain your work, to make it clear for new residents and you are so great to make it fun! Thank you very much for all the personal and professional support during these 4 years.

I really want to thank Luc Duchateau for his invaluable help in the statistics of all articles. Thousand times thank you for your patience, your availability, your explanations, your corrections and your support, keeping a good mood whatever the stupid ideas I can tell you, during these 4 years.

Je voudrais remercier Laurent Marescaux pour avoir accepté de participer à ce comité d'examen. Merci mille fois aussi pour tous tes conseils avisés lors de mes stages à Oncovet, d'un point de vue radiologique, comme professionnel de manière plus générale. Ils m'ont été tellement utiles depuis, au moment de l'examen comme dans mon nouveau poste de radiologue. Ce fut un réel plaisir de travailler quelques temps avec toi.

Un grand merci à Fabrice Conchou pour avoir accepté d'être dans ce comité d'examen et de venir de si loin pour la défense. Merci de ton soutien et de tes encouragements. Tu m'as toujours impressionnée par ta persévérance malgré

la difficulté et les obstacles. Ne lâche rien et on aura très vite un diplômé ECVDI à Toulouse !!

I would like to thank Pr Van Bree for giving me the opportunity to do the residency and the PhD in the medical imaging department of the veterinary faculty.

A great thanks to all the co-authors and reviewers of the articles in this PhD for their help and advice to built the project. I am so grateful to Sylvie Daminet, Dominique Paepe and Dan Rosenberg, for their precious guidance in the internal medicine topics of this thesis.

I would like to thank very sincerely Ingrid P for her commitment in the resident training and for all the professional and personal advice. Thanks a lot for your constant help and support, your knowledge you want to share, all the stories you told us, the clinical sense you want to teach us. And the most important here, a very very very great thanks for the feline adrenal impulse!!!

I would like to thank particularly all the residents I worked with during the 4 years in Ghent and abroad, for all the help, the mental support and the fun moments. Part of the training consisted in sharing advice, ideas and knowledge from everyone: to study, in clinic and in research. Being always a lot of resident in Ghent was a real chance for me: big hug to Elke VdV, Elke S, Hendrik, Yseult, Pascaline, Olga, Veerle, Caroline, Laure, Els and Emmelie. A great thank you to Anne-Sophie, Penelope, Kerstin, Nele, Lise, Frederic, Nathaniel and Guillaume. Never give up !!!!!!!!!!!!!!!

Elke VdV: a very very big thanks for all the support and the advice you gave me during your residency and after. Thank you for your patience when I was stressed and jumping everywhere from one thing to another.

Laure: une autre grande découverte de ces années de PhD... mais humaine celle-ci ! Ton pétillant parfois explosif, ta motivation canon, tes attentions permanentes, nos séances sportives à refaire le monde, tes recettes, etc. tout ça me manque déjà. Merci pour ton soutien et ton aide dans les moments difficiles. Le début d'une grande amitié, j'espère...

Olga: thank you for these years we worked together, I really discovered a great colleague, who opens my mind and my mood, I hope we will keep in touch for long. I wish you to return soon to real life with a nice piece of paper...

Caroline: merci mille fois pour ces deux ans de colocation !!! Nos conversations sur la condition humaine comme l'analyse de Grazia vont me manquer. Ne lâche rien Caro, et surtout surtout je te souhaite tout le bonheur du monde.

Veerle: thank you for sharing these years of work, sharing the bad as much as the good mood. I wish you all the best: you deserve it so much!

Els: thanks a lot first for the welcoming in 2009, it was so nice to meet kind colleagues like you when I arrived in the office. Thank you for all the scientific and humane exchanges during these 4 years. A special equine thanks!! I wish you all the best with the residency, I am so impressed by your work, with family, clinic and study: don't give up and success!!

A special thank for all my colleagues during these 4 years in the medical imaging department who helped me building my experience in this field: Ingrid G, Annemie, Kaatje, Casper and Walter in CT/MRI; Kathelijne, Simon and Eva in Nuc Med; Kees Dik, Sarah, Stijn, Els and Katrien in Large Animal Radiology. Thanks a lot for all the clinical work together, all the invaluable film reading sessions and all the shared knowledge.

A great word of thanks for all the clinicians in the Ghent veterinary hospital who encouraged me to become a better radiologist, with all the nice cases and all the knowledge we could share for 4 years. Thank you also for enduring me the bad days! Special thanks to Dominique P, Miguel, Alenka, Barbara, Ine and Alejandro. A little word to the 4 generations of interns who worked in Ghent with me: great job and thank you! I also would like to thank all the equine clinicians, very patient with me and helpful in my equine training in radiology. Finally, I want to thank the orthopaedic team, for all the work together, in the good and the bad days.

A great thanks to all the co-workers in the medical imaging department: Marleen, Marnix, Kim, Tom, Claudine, Natasja, Sandra and Stijn. Thank you for helping us running this department. Thanks a lot for solving all the technical, radiological, computer-related, network-related, administrative, financial issues everyday!

Mes amis et ma famille sont mes moteurs, je n'en serais certainement pas là sans eux. Je ne trouve même pas de mots adaptés pour les remercier suffisamment. Un gigantesque, énorme merci pour votre soutien sans faille

depuis 30 ans, dans toutes ces épreuves. La prochaine étape : passer pas mal d'année tout près de vous et partager encore plus de Bonheur.

Last but not least, je remercie l'amour de ma vie, qui m'encourage chaque jour, me stimule dans les bons comme dans les mauvais jours, me donne confiance, me guide au quotidien en préservant mon individualité et surtout qui m'a attendu chaque jour de ces 4 années. Merci Michaël d'être un homme parfait. Mon plus grand bonheur est d'être à tes côtés tous les jours.

“Cherchons comme cherchent ceux qui doivent trouver et trouvons comme trouvent ceux qui doivent chercher encore. Car il est écrit : celui qui est arrivé au terme ne fait que commencer” Saint Augustin

